THE PRINCIPLES AND METHODS OF PHYLOGENETIC SYSTEMATICS AND ITS APPLICATION TO THE TAXONOMY OF THE PRONOCEPHALIDAE LOOSS, 1902 (PLATYHELMINTHES : DIGENEA)

A thesis

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PREFACE

A Personal Introduction

If I were asked to describe that period between 1987 and 1989 when I worked for my Ph.D., I would say that it was stimulating and exciting - in short, I would say that I enjoyed myself. Working in the area of theoretical and applied systematics has allowed me to combine three fields of study of which I am particular fond - mathematics, computing, and evolutionary biology. It is with this feeling of excitement in mind, that I have chosen to present not only the results of the systematic research I have carried out, but also the motivation for such research.

More often than not, theses and journal articles, constrained as they are by tradition and editorial policy, are sterile reports of scientific work. They fail to reflect the strong personal component in all scientific enterprise which, to a large extent, determines its direction. While personal reflections, such as those which I have included in this thesis, may be considered irrelevant by some, they provide the backdrop against which this work is set, the context in which it can be understood. Aside from answering the *scientific* questions which I have attempted to solve, this thesis also addresses the *personal* questions, "Why did I do such-and-such ?", and, "What do I really believe in ?".

A Ph.D. is almost a kind of Membership Card into a community of scientists, and I believe that it is as important to understand the person as it is to acknowledge the work, before membership is granted.

Although my work on the systematics of the Pronocephalidae began in 1987, my interest in phylogenetic reconstruction and cladistics was sparked off by the visit of Dr. Dan Brooks, in 1986. Dan is a vital and enthusiastic individual, and his enthusiasm is contagious. In 1987, then, when I began to work on revising the taxonomy of the pronocephalids, under the supervision of Dr. David Blair, I was sure that there was one and only one reasonable way to reconstruct evolutionary history - parsimony.

I soon realised that there were gaps in the techniques available, gaps which I had to fill myself. As the work progressed, it also became apparent that the fundamental assertions of cladists - to a large extent, these are philosophical - are open to debate and subject to criticism. At this stage, I thought it necessary to review these claims, for my own satisfaction. I was loath to doggedly follow a course of action until I was satisfied that its principles rested on firm foundations. Hence, my brief sojourn into philosophical realms.

The three parts of this thesis is a reflection of these three periods of my research, although the order in which the first two parts are presented is the reverse of the chronological order of the work.

Most of my results have been written up as manuscripts and submitted to various journals for publication, or presented at conferences. I have treated these as chapters of my dissertation. Although each chapter has a different emphasis, there is often some overlap between chapters. I ask the reader to bear with this. In each chapter, I have also included, when necessary, footnotes, and addenda.

Allen Rodrigo
April, 1990

ABSTRACT

Biological systematics has developed according to the Kuhnian model of science: there have been paradigm shifts in systematic practices, the consequence of changing perceptions of what is required of scientists, theories, and classifications. Under the current paradigm of phylogenetic systematics, there are two sub-disciplines which can be broadly categorised as Methodological Procedures and Modelling Tools. The former include the techniques of Parsimony and Compatibility, while the latter consists of the recently developed techniques of Maximum-Likelihood Estimation (MLE). Parsimony and Compatibility, while intuitively appealing, can lead to incorrect hypotheses of phylogeny when characters of taxa change at unequal rates. MLE takes account of unequal rates of change, but is mathematically demanding. In this thesis, a number of methods are derived which retains the simplicity of Parsimony and the efficiency of MLE. These techniques are applied to uncovering the relationships of the Pronocephalidae Looss, 1902 (Platyhelminthes:Digenea) a family of monostomatous parasites of reptiles. The analsysis revealed that certain genera of the Pronocephalidae are polyphyletic. In revising the taxonomy of the group it was necessary to erect a paraphyletic genus because of the insufficiency of good character-taxon information. Under the revised classification, the Pronocephalidae consists of seven genera: *Notocotyloides* Dollfus, 1966, *Pyelosomum* Looss, 1899, *Charaxicephalus* Looss, 1901, *Pronocephalus* Looss, 1899, *Cetiosaccus* Gilbert, 1938, *Macravestibulum* Mackim, 1930, and *Neopronocephalus* Mehra, 1932.

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PART I

THE PHILOSOPHY OF SYSTEMATICS

"Philosophy ... is not a presumptious effort to explain the mysteries of the world by means of any superhuman insight or extraordinary cunning, but has its origins and value in an attempt to give a reasonable account of our own personal attitude towards the more serious business of life."

Josiah Royce

The Spirit of Philosophy

"Every genuine scientist must be ... a metaphysician"

George Bernard Shaw

Back to Methuselah

INTRODUCTION

As a group, scientists are reticent about engaging in philosophical debate. Science, after all, is in the business of finding out empirical truths. The point and counterpoint arguments of philosophers, the endless dialogues on metaphysical abstractions and semantic minutiae, have no place in scientific enterprise, for they cannot be resolved objectively by recourse to Hard Fact.

Many scientists would go further and say that the converse is equally true: science has little or nothing to gain from philosophical soul-searching. This is patently false. Logic is the yardstick by which we measure the quality of our hypotheses and theories, inferences, and experiments. Theories about knowledge and the acquisition of knowledge -the domain of epistemology - allow us to evaluate the soundness of our scientific methods. We cannot reasonably expect to explore the boundaries of our concepts, and the adequacy of their definitions, without recourse to some form of metaphysical discourse.

The disputes that currently rage in the diverse field of evolutionary biology typify our reliance on ideas philosophical. The debates between Creation "Scientists" and Evolutionists, Structuralists and Functionalists, Pheneticists and Phylogeneticists, are, in essence, philosophical dialogues.

I have always had a passion for philosophy, particularly the philosophy of science. However, this section of my work owes more to my bewilderment at the level of disagreement between systematists over fundamental issues, than any self-indulgence.

As I mention in the Preface, I began this project believing strongly that cladistics was the panacea for all the ills of biological systematics. It was only after I had applied parsimony techniques to real data sets, that I began to see some of the shortcomings of the method. In the next section, I will review these problems and offer some solutions. However, more often than not, the solutions that I had developed altered cladistic methodology to such an extent, that it was "parsimony" in name only. It was this fact that led me to a closer inspection of the aims, and foundations of parsimony as a technique of phylogenetic systematics. (A note on terminology: I use the term *cladistics* to refer specifically to the method of constructing phylogenetic hypotheses using parsimony. *Phylogenetic systematics*, on the other hand, is a generic term referring to all methods that are designed to reconstruct evolutionary history: these include parsimony, compatibility, and maximum-likelihood estimation).

In the two chapters in this section, I will confine myself to an examination of the differences in contemporary systematic methods, and the extent to which they are reconcilable. Which, if any, is the "best" method of phylogenetic reconstruction? More specifically, I have directed my efforts at refuting the cladists' claim that their choice of technique, parsimony, is the "best" method. I argue that parsimony lacks the philosophical endorsement that its proponents claim, and that indiscriminate use of the technique is unwarranted.

There is an obvious bias in my assessment of the techniques discussed and it stems from the fact that my work is primarily concerned with the application of systematic methods to *taxonomic* research. To an extent this is unusual, for in the past two decades there has been a growing conviction among systematists that the most interesting research - that which deserves the most attention - involves the reconstruction of the evolutionary histories of groups of organisms *per se*. Taxonomic revision has become almost incidental. For example, in *Systematic Zoology*, from 1978-88, 22 papers that considered taxonomic relationships among various groups were published. The authors of only five of these took that extra step and proposed revisions of the taxonomy of the respective taxa. What then of the taxonomist whose principal research involves the necessary and difficult tasks of classification and identification? Does the New Dawn of Systematics shine on him/her ?

I have approached systematic research with the following question in mind:

Given the constraints inherent in taxonomic research, which systematic method (or suite of methods) allows the taxonomist to construct the best possible classification ?

All practising taxonomists will be familiar with the nature of these "constraints": the lack of live or type specimens, the obvious requirement for a single, preferably stable, classification (as opposed to a number of alternative hypothetical hierarchies), the need to work within the guidelines of the International Code of Zoological Nomenclature, and the recognition that classifications are more than a codification of genealogical hypotheses - they perform a service to biologists and biological resource managers, by providing a database of biological information.

In the following papers, *taxonomic efficiency* is the principal criterion by which I judge cladistics, and other systematic methods.

CHAPTER I

THE KUHNIAN STRUCTURE OF BIOLOGICAL SYSTEMATICS

A paper presented at the Arthur Prior Memorial Conference, a Joint Meeting of the Australasian Society for Logic and the New Zealand Society of Philosophy, 1989, held in Christchurch, New Zealand

"Here is the beginning of philosophy:
A recognition of the conflicts between men,
A search for their cause,
A condemnation of mere opinion ... and the
discovery of a standard of judgement."

Epictetus
Discourses, Book II

incommensurable *a.* (Of magnitude) having no common measure integral or fractional (*with* another);

The Concise Oxford Dictionary

I have a practical interest in philosophy: what I ask of philosophy and philosophers is, "How can I understand the development of my science, and to what extent can I use this understanding to examine the different theories with which I am confronted?". I am particularly drawn to the ideas of Thomas Kuhn and in this paper I contend that the development of biological systematics, can best be understood in the light of Kuhn's model of science (Kuhn, 1970).

This paper consists of two main sections. First, I consider the practical consequences of Kuhn's model of science, and how these differ from the received view of science. Second, I will show how Kuhn's model can be applied to the development of biological systematic research.

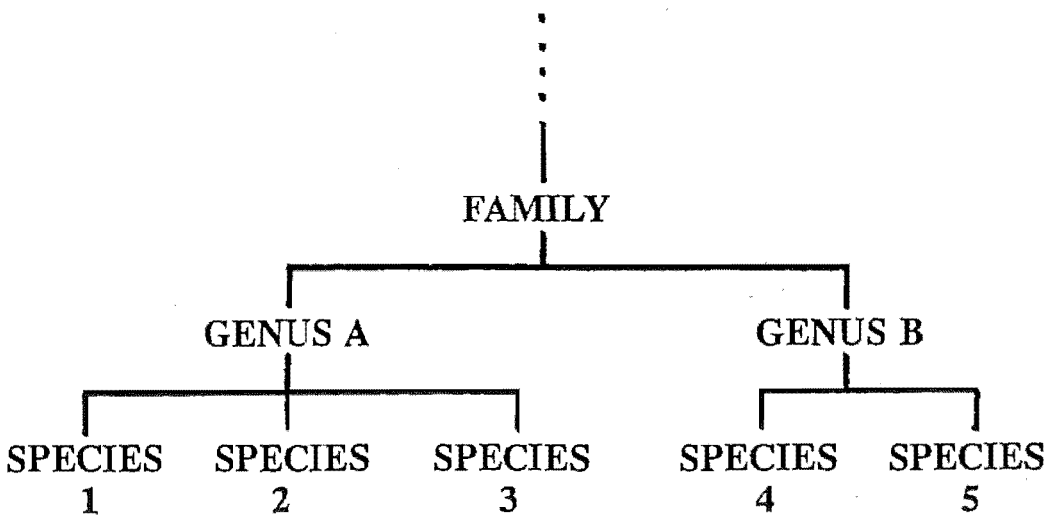
Biological systematics is concerned with the organisation of biological information - information derived from groups of related organisms (Abbott *et al*, 1985). Such information typically takes the form of "attributes" or "characters" of these groups of organisms, although it may be extended to include theoretical information such as "evolutionary relationships". For instance, I may say that the species *Felis domesticus* is a quadripedal carnivore with well-developed tympanic bullae, orbits, and retractile claws, and in doing so, I would have provided biologists with some of the information needed to identify the household cat. Characters include information on anatomy, ecology, dietary habits, geography, and behaviours of the group in question. Also, by virtue of its proper name, biologists know that the household cat is related to other members of the Felidae - lions, tigers, panthers, leopards, etc. Now, I will avoid the obvious problem of proper names and what they refer to, and state that, in the main, biologists use species names in the sense of Frege (see Dummett, 1981), that is, names of groups of organisms carry with them a baggage of descriptions, and properties.

One of the primary goals of systematic research is the production of classifications - schemata which provide some way of retrieving character-information, and which also identify the relationships between the groups of organisms. I will leave the term "relationship " undefined for the moment, and note only that relationships are inferred from characters and are therefore theoretical constructs and not empirically observable.

Typically, classifications have the following formal structure, developed by Linnaeus and others in the middle 18th century, and remarkably well conserved (Fig. 1.1):

The structure is hierarchical, with the lowest level representing relationships between species or sub-species. In Fig. 1.1, Species 1, 2, and 3,

Figure 1.1 A schematic representation of the Linnean hierarchical system of classification. The lowest major category is the species. The other major categories are the genus, family, order, class phylum, and kingdom.



are more closely related to each other than any is related to Species 4 and 5. The major categories are the *genus*, *family*, and on it goes until we have the *kingdom*, traditionally separated into the *plant* and *animal kingdoms*. [Note: Nowadays, Whittaker's (1969) five kingdom classification is more generally accepted].

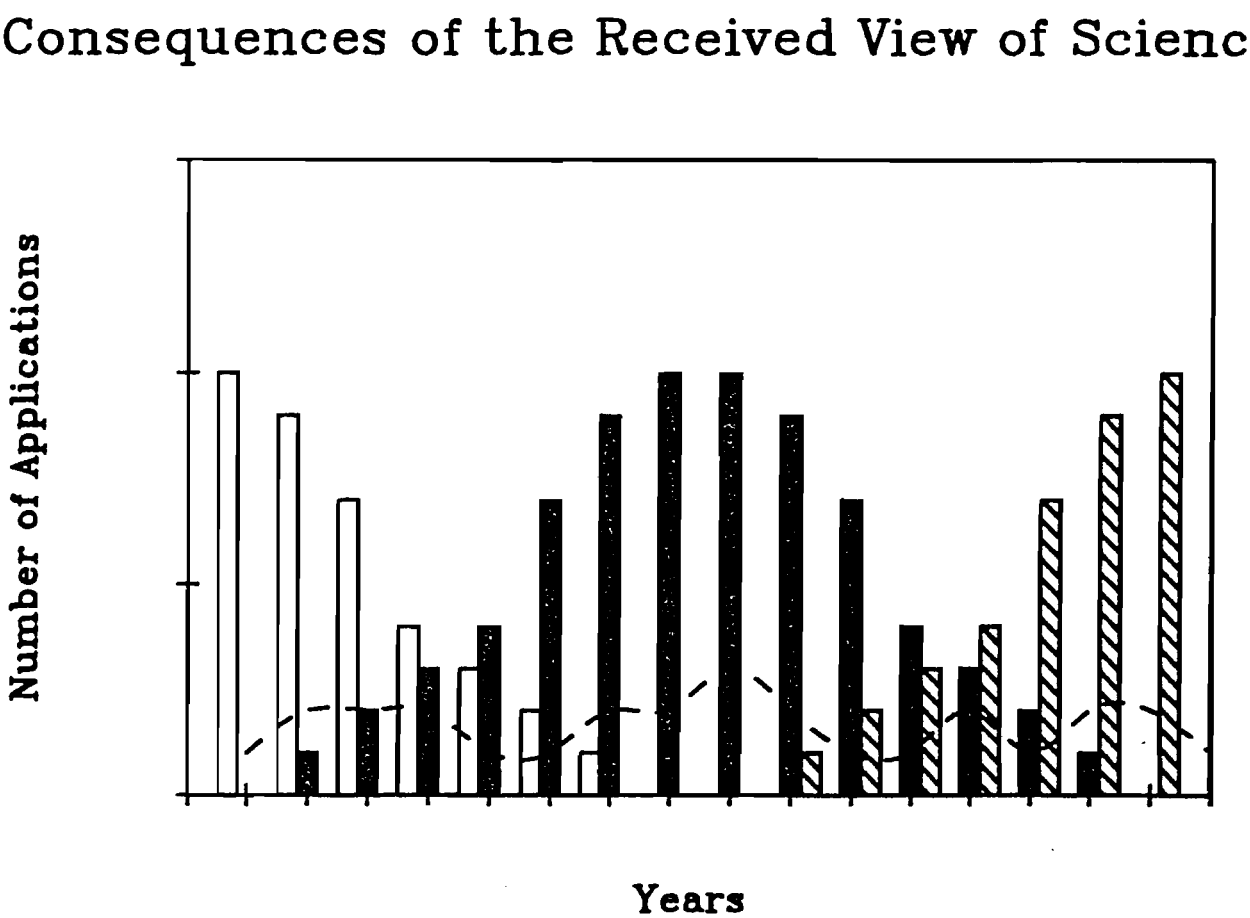
I think it is important to realise that classifications are structurally and functionally equivalent to scientific theories. They are constructed in the same way, and serve the same purposes as more familiar theories of science, i.e., they play both a predictive and explanatory role. Once again, I will defer my discussion of the théoretical nature of classifications, because it is tied in closely with the notion of "relationships".

Having briefly outlined the goals and "subject matter" of systematics, let me now turn my attention to Kuhn and his view of science.
It will be helpful to identify the main points of Kuhn's view of science, as outlined in his *Structure of Scientific Revolutions* (1963; with Postscript, 1970), and to contrast these with the so-called Received View of Science. Hilary Putnam (1962) coined the phrase "Received View" to refer to the formal characterisation of scientific theories as axiomatic, or more correctly, axiomatisable systems, a view that was popular in the 1920s and 30s. Since the inception of these ideas, the Received View has undergone a number of changes, which are reviewed by Suppe (1979) in *The Structure of Scientific Theories*. However, proponents of the Received View still hold the fundamental position that scientific theories are axiomatisable systems.

What does the Received View say about the development of science? According to its proponents, scientific theories arise by a Baconian process of accumulation of data, hypothesis testing, empirical corroboration, and subsequent acceptance. Scientific theories may cease to be accepted because of their inability to explain novel phenomena, or they may be absorbed into other theories that encompass a wider range of phenomena. The acceptance and rejection of any one theory is an objective decision based on the ability of that theory to explain and/or predict new phenomena. Furthermore, new and old theories share the same *observational* vocabularies, i.e., terms assigned to observations maintain their meanings through time. *Theoretical* terms change only in the sense that they become generalisations in which referents of such terms (i.e., entities to which these terms refer) in older theories are special cases.

It is interesting, that laypeople, most scientists, and some philosophers accept that this is the way science really progresses. But what are the practical consequences of such a view of science? What can we expect to

Figure 1.2 The practical consequences of the received view of science. The applications of three different theories are represented by the different bars,(□), (■), and (▨). The applications show a cyclical trend: as theories lose their ability to explain natural phenomena, the number of applications which rely on these theories decline in number. Successive applications are based on newly developed theories which are able to explain the "anomalies". The dashed line indicates the level of philosophical discourse. This remains relatively constant, since most of the concepts and terms remain fixed.



see in terms of the applications of scientific theories to real-world problems?

Certainly, we can expect to see a cyclical trend in theoretical applications as old theories lose their applicability - either by a decline in predictive efficiency, or an inability to explain new phenomena -and new ones gain acceptance. Furthermore, when a new theory becomes acceptable, we can expect that there will be no applications which resort to the explanations or predictions of the old theory. And finally, because descriptive terms retain their meaning in the face of theory change, we do not expect any changes in the level of philosophical discussions involving, among other things, meanings of observation and theoretical terms. These consequences are illustrated in Fig. 1.2.

Now, in contrast to the Received View, Kuhn maintains that theories are problem-solving structures that cannot be reduced to axiomatic systems. They are devices moulded by a community of workers bound together by a constellation of beliefs, values, and concepts (collectively called the *disciplinary matrix*). In other words, there is a strong sociological component in scientific theories. If we are to use theories, as scientists do, we cannot disentangle them from this social component, and we cannot understand them in isolation from the community that uses them. Kuhn does not claim that there is no empirical input into the formation and acceptance of theories. Rather, there is no way we can deconstruct a theory to reveal exactly what is empirical and what is sociological.

Kuhn uses the term *paradigm* to identify both the disciplinary matrix of a scientific community, or sub-community, and *exemplars*, which seem to refer, in Kuhn's sense, to the *kinds* of problems for which solutions are commonly required. In other words, exemplars serve as informal "pointers" to the problems which members of a scientific community are directing their attention. Now, the disciplinary matrix plus the exemplars of a particular sub-science make up what I will call the research programme. By my definition, then, a research programme is a directed protocol for problem solving.

What is a scientific theory? Is it a propositional component of the disciplinary matrix - a sub-paradigm? Or is it the totality of disciplinary matrix and exemplars? Kuhn argues that it is the latter. If scientific theories are problem-solving devices, then they must be equivalent to the research programme, for it is only from within that structure that problems are solved[1].

Obviously, It is difficult when one attempts to compare scientific

theories in Kuhn's model with those of the Received View; I have attempted to avoid the problem in this paper, by allowing the terms to be contextually defined.

In brief, what, then, are the elements of Kuhn's view of scientific development (Box 1.1)? Periods of normal science, in which a paradigm is used by most members of a scientific community to solve problems, alternate with revolutionary periods, which result in changes of paradigm. Pre-revolutionary periods are characterised by the emergence of anomalous situations - phenomena which cannot be accounted for by existing paradigms. The crisis of recurrent anomalies leads to the emergence of competing paradigms (which I will equate with research programmes). Competing paradigms exhibit incommensurability, and they do so because of their very structure.

Kuhn does not give a completely satisfactory definition of incommensurability. It seems reasonable to me that the notion of incommensurability can be divided into three categories, each representing a level of potential for reconcilability of competing research programmes. I do not however, envisage that these are discrete; on the contrary, they flow into each other and can be arranged on a continuum.

Weak incommensurability arises when scientists disagree only with respect to notions of utility, value, fruitfulness, simplicity, efficiency, etc., of methods. In other words, there are no real conceptual differences, insofar as theoretical and observational terms go. More often than not, these differences are manifest in applications of different methods or techniques. For example, biologists often speak of populations of organisms, and in many cases biologists recognise the same real-world entities as populations. However, one biologist may choose to measure population size by estimating the total weight of all individuals in the population, whereas another may choose to determine population size by estimating the total number of organisms, because it is easier.

Strong incommensurability involves differences in meaning and reference. Quite often these differences are obscured: scientists from competing paradigms are not aware that differences exist. Consider a real example. Studies of energy flow between trophic levels require that population size be measured in biomass or, better still, energy equivalents. However, in order to solve population dynamic problems such as population growth, the usual practice is to use the number of individuals as a measure of population size. Inter-relating the results and theories of these studies requires that there is an understanding of these differences. That

Box 1.1 Elements of Kuhn's model of scientific development. See text for elaboration.

ELEMENTS OF THE KUHNIAN DEVELOPMENT OF SCIENCE

1. Normal Science and puzzle-solving.
2. Anomalies and crises.

3a. The emergence of competing paradigms (=research programmes).

3b. The incommensurability of competing paradigms:
   - weak incommensurability
   - strong incommensurability
   - very strong incommensurability.

4. Revolutions, characterised by:
   - increase in philosophical discourse
   - political conflicts
   - prospective invisibility.

5. Post-revolutionary periods, new paradigms, and retrospective invisibility.

differences exist is often obscured by the fact that the same terms are used in both research programmes. Strong incommensurability is strong precisely because the differences in meaning are transparent, and the recognition that differences *do* exist comes much later.

Finally, there is, what I call *very strong incommensurability*. This occurs when competing paradigms require a major shift in perception, the change in the *Weltenschauung* of alternative research communities, what Kuhn calls a Gestalt-switch. This would occur if, for example, a new research programme emerged in which it was claimed that populations are unnecessary abstractions, and only individual organisms exist. The analogy is one which Kuhn uses: different languages are spoken, and translation is the only recourse.

Darwin (1859) initiated just such a change in biology by suggesting that humans had not arisen by some act of special creation but are products of the same evolutionary processes that have given rise to gnats, gannets, and gorillas. There are a number of consequences which follow a Darwinian view of the world. For example, the realisation that other species have as much right to be here as our own lends credence to the aims of the conservation and anti-vivisection movements. On the negative side, the fact that human behavioural and psychological profiles have been shaped by natural selection has been used by some to reinforce notions of Caucasian superiority.

Scientific revolutions occur when there is strong incommensurability between competing paradigms. As Kuhn notes, such revolutions are often characterised by an increase in philosophical discourse, particularly about things conceptual: the ontology of theoretical and even observational terms are questioned; and often there are long diatribes about whether one method is "more scientific" than another. Political conflicts occur and these are often manifested as "authority bashing".

Apart from the incommensurability of competing research programmes, Kuhn also devotes a section of his book to the notion of *invisibility*, as it pertains to scientific revolutions. He is referring more to *retrospective* invisibility: the rewriting of history, as it were, to portray science as an accumulative process. Scientific revolutions are also *prospectively* invisible, however, i.e., scientists seldom know that they are moving into a period of revolution. In part, this is because most competing paradigms are perceived as solutions to the same kinds of problems which faced the old research programme. Only after some intense conceptual analysis, do proponents of the new view realise that there has been a

paradigmatic shift.

In the post-revolutionary period, scientists settle down to work under the new paradigm, except that there may be a residual adherance to the old ways of science. In Kuhn's model, old research programmes never die, they just fade away.

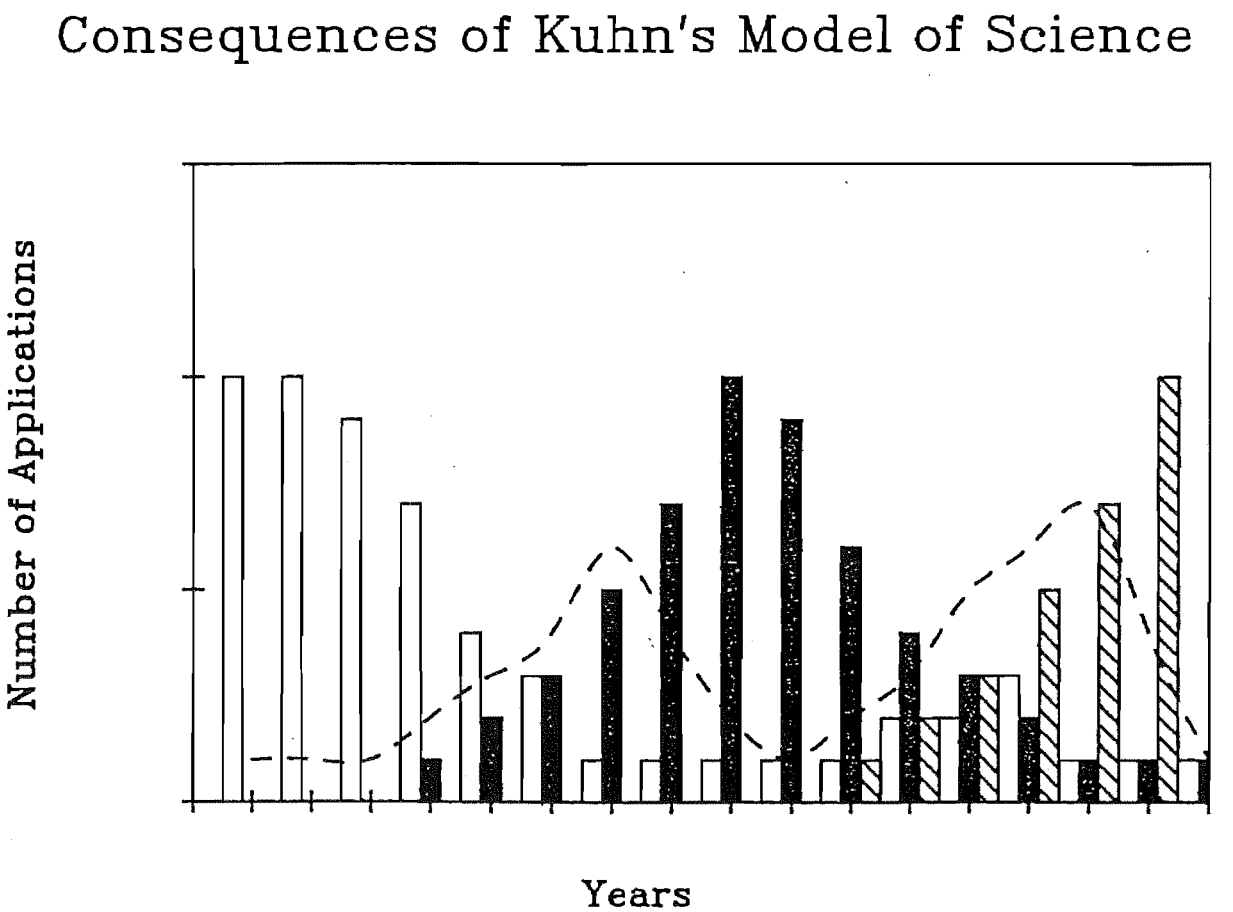
What then are the practical consequences of Kuhn's model of science, and how do these contrast with those of the Received View? In my opinion, the major difference is the fact that traces of old research programmes remain. "Diehard traditionalists" still apply methods that have been forsaken by the proponents of contemporary paradigms. However, whenever there is a revolution, traditionalists may attempt to put their view forward as a viable alternative (often prefacing their remarks with a less ascerbic equivalent of "I told you so"). Also, there is a definite pattern to the levels of philosophical discourse: at times of crises, these begin to rise, and at the emergence of a new paradigm, they begin to subside (see Fig. 1.3).

These then are the practical consequences of both the Received View and Kuhn's model of science. How does the history of biological systematics compare with both of these models? We can identify three different systematic research programmes. Two of these - synthetic systematics[2] and phylogenetic systematics - hold that classifications should reflect evolutionary relationships. In contrast, proponents of phenetics maintain that overall similiarity between groups determine relationships. The term *relationships* has different meanings when used by different groups or "communities" of systematists, giving rise to a potential for incommensurability.

Apart from just differences in meaning, a number of other factors interact to give rise to the incommensurability between the three competing systematic research programmes. I will examine two of these factors here. They relate to different concepts of species, and different classification-construction methods and criteria.

First, consider the species concept, which, incidentally, has been one of the most troublesome of biological concepts, the ontology of which no one seems sure of; as a result, it spends its time flitting from being an observable entity to a theoretical entity, to a non-entity. Synthetic systematics inherited the species concept of the systematics before the New Synthesis. It was not really a concept; it was more a way of applying species names. The biological community relied on and trusted taxonomists to give names to things that had some measure of spatio-temporal stability with respect to the attributes associated with these things; this was the way

Figure 1.3 Consequences of Kuhn's Model of Science. Again, the different bars represent the applications of different theories (see caption to Figure 1.2 for key). Unlike the Received View, however, applications of theories which have become unpopular are never completely absent. Instead, there remains a residual level of applications founded on these theories. Furthermore, Kuhn predicts a fluctuating level of philosophical discourse (represented by the dashed line), as the meanings of theoretical terms change, and become incommensurable. These philosophical debates are more intense at the period of scientific revolution and theory change.



taxonomists worked (Mayr, 1969:5-6). With the New Synthesis, it was believed that there should be a formal definition pinning down the concept of species, and that taxonomists should try to identify species by their correspondence with the terms of this concept. In addition, it was felt that the species should be given a definition compatible with its role as the unit of evolution. Hence, a species was now considered to be potentially interbreeding populations that were reproductively isolated from other populations. In effect, synthetic systematics now encompassed two (not always compatible) species concepts - the *taxonomic species*, which would not go away [see, for instance, Blackwelder's (1967) defence of the taxonomic species], and the *biological species*.

The question that must be asked is "Why the insistence on potential interbreeding?" That it is a response to the genetics of the New Synthesis is only a partial answer. Another aspect of the answer can be found when one considers the person who is, arguably, the most influential initiator of the change: Ernst Mayr.

Mayr has been and still is one of the leading evolutionary biologists of this century. It was he who first gave a plausible definition of a Biological Species (Mayr, 1942). Mayr is an ornithologist, and since birds interbreed, it seems only natural, I think, that Mayr should propose this particular definition of the species[3]. However, it is nonetheless true that the biological species concept leaves out a large proportion of animals and plants which are asexual. What is quite amazing, though, is that the definition became accepted as <u>the</u> definition of the species for more than a quarter of a century, and is still widely accepted today (for example, refer to any general biology textbook).

Dissatisfaction with the biological species concept grew, not only because of its lack of fit to the "real world", but also because it was notoriously difficult to apply (Heywood, 1984). Taxonomists are usually unable to tell if populations are potentially interbreeding or reproductively isolated. Ultimately, a competing view of species emerged; but the biological species concept did not die out. Many biologists still hold to the biological species concept, even though Mayr amended his view to the extent that it is impracticable in almost every instance (Mayr, 1982; Rosenberg 1985).

The emergent view of the species in 1950s and 60s was that of the *phenetic species* or *phenotypic species* as it is sometimes called. The idea of the phenetic species ties in closely with the methods of phenetics and I will deal with it only briefly. Essentially, it was a rebellious response to the attempt to identify the species as the unit of evolution (Sokal and Sneath,

1963). Pheneticists rejected the notion that we can "see" evolution and evolutionary units. They felt that taxonomic units should be defined so as to provide biologists with the greatest amount of information about the attributes of the individuals making up these units. To pheneticists, the species is a taxonomic unit like all other taxonomic units, except that it is the lowest such unit (Rogers and Appan, 1969; Doyen and Slobodchikoff, 1974). Now, applying the phenetic species involves adhering to a radically different view of the world, and the function of systematics. The phenetic species does not have the shortcomings of the biological species concept, in that it can be easily applied to all groups of organisms.

However, for ontological rreasons the phenetic species has ceded its place as the natural species concept to the *evolutionary species*[4] (Simpson, 1961; Wiley, 1981), and this occurred because of ontological reasons. It was claimed that the phenetic species (and all phenetic taxa) was not "real" (or natural), because it did not reflect the process of evolution (Wiley, 1981). But why should taxonomy reflect evolution? The main reason is the belief that biologists, by virtue of their work, are best served by classifications which reflect evolution (Cracraft, 1983). But many contemporary microbiologists and geneticists are quite comfortable with phenetic classifications, as are many botanists (Abbott *et al*, 1985). There is no real evidence, then, that biology is better served by one system than another. However, the revolution occurred, and the evolutionary species concept is now widely accepted. However, like the biological species concept, the phenetic species concept persists.

The evolutionary species concept includes the biological species concept as a special case. It is an attempt to mirror reality - or reality as viewed by the phylogenetic systematists. There are many problems with the evolutionary species concept - what is a historical lineage, and how do we infer evolutionary isolation - but it is quite acceptable to most contemporary systematists ... for the moment.

Of course, throughout the evolution of systematic research programmes there have been other species concepts jostling to emerge. The most recent one (the Self-Defining Species Concept[5]) paints what is perhaps the best picture of incommensurability between paradigms. Consider the following quote from Lambert *et al* (1988), writing in its favour:

> "... we intend to concentrate on a discussion of biological species concepts and to identify one that uses truly biological criteria for species definition."

This "one" is the self-defining species concept; but the point is that proponents of every species concept claim that their concept is founded on

truly biological criteria. Obviously, the terms that are being used mean different things to different biologists.

Let me now turn my attention to the classification methods of different research programmes (a more complete account is given by Hull, 1988). Synthetic systematics developed methods that purported to construct classifications reflecting evolutionary relationships. Such classifications are theoretical structures which explain similarity of form and function among related organisms. However, much intuition and experience was involved in the building and acceptance of these classifications. For instance, it was accepted practice for systematists to decide which characters were good indicators of phylogenetic relationships (= homologies) and which were not (Cronquist, 1969).

The transition from synthetic systematics to phenetics was brought about primarily by dissatisfaction with what were perceived as the subjective methods of synthetic systematics. It was held that since we can never test classifications that reflect evolutionary relationships (Sneath, 1983), there is no necessity to view evolution as the guiding light in the construrction of classifications. Structural resemblance was considered the appropriate measure of relationship, and resemblances were measured statistically.

Who were the people who instigated the phenetic revolution? By and large, they were statistically-literate biologists. Robert Sokal and James Rohlf, two pioneering pheneticists, are also biostatisticians. They introduced into statistical methods into systematics, and a Fisherian way of looking at things [for instance, Sokal (1983) characterises the search for a hypothesis of relationship as an estimation of the population classification from a sample estimate]. Quite often these numerical methods did not suit the fuzzy nature of biological form, but they were applied nonetheless. They were applied to such characters as colour and shape; and it was considered acceptable to do so (Holling and Stace, 1978). It should be noted that many biologists who adopted the techniques of phenetics still accepted the concepts of the old synthetic methods. What attracted them to phenetics was its so-called objectivity. However, relationships were still thought of in the context of evolution (e.g., Suh *et al*, 1974). To these systematists, phenetics was just a new way of doing things. In effect, many systematists entered the systematic revolution without knowing it was there - an instance of prospective invisibility.

Of course, it *was* a change in world-view, but it took a great deal of philosophy to make biologists realise this. Once biologists understood that

phenetic relationships were not evolutionary, dissatisfaction returned, and the scene was set for a change to phylogenetic systematics, and the return to methods that attempted to reflect evolution (Heywood, 1984).

Phylogenetic systematists base their classifications on common ancestry - groups are closely related if they have a recent common ancestor. Their methods remain mathematical but instead of statistical methods, they use graph-theoretic methods[6]. One of the main features of phylogenetic systematics is the fact that all characters are assumed, *a priori*, to be contingent on ancestry (see Addendum 1.1 to this chapter). This assumption does not satisfy the synthetic systematists, however, because they place great emphasis on differences between homologous and analogous characters. Why then did phylogenetic systematics become popular?

One reason is that systematists were unwilling to return to the subjective method of synthetic systematics. Furthermore, phylogeneticists supported their methods by aggressively claiming philosophical superiority. As a group, the phylogeneticists are Popperians (Wiley, 1981). They have convinced - and "convinced" is the right word - most biologists that the Popperian criterion of falsifiability is the mark of a true scientific theory. They maintain that their classifications meet this criterion. If one rejects falsifiability, then one is being unscientific.

Phylogeneticists have changed our view of the world yet again: characters are treated as homologous traits, unless proven otherwise; Science proceeds by the development of falsifiable theories; and observations of characters and attributes are theory-independent (or relatively so).

Now, in every period of crisis and revolution in biological systematics, a mass of philosophical papers have appeared, all devoted to comparing classifications in the light of different values. These values include:

Informativeness. Phylogeneticists have been criticised for erecting classifications that ignore the degree of character divergence - information which the synthetic systematists feel is very important (Faith, 1983). Both the phylogeneticists and the synthetic systematists criticise the pheneticists for ignoring evolutionary information. The pheneticists rebutt by claiming that the other methods tell biologists little about the characters of taxonomic units (Colless, 1981).

Naturalness. Pheneticists claim that their classifications are natural because they contain information about the nature of the organisms (Sokal, 1983). Phylogeneticists claim that their classifications are natural because they reflect a natural process - namely, evolution (Funk, 1983).

I have shown how the history of systematic research reflects the main

tenets of Kuhn's model of the evolution of research programmes. Both Kuhn's model and that of the Received View are also amenable to *empirical* corroboration by an examination of the pattern and number of applications of systematic methods through time. Figures 1.4 and 1.5 illustrate this, and may be compared with the predictions of the models of Kuhn and proponents of the Received View, in Figs. 1.2 and 1.3, respectively. The data I collected consist of the number of papers appearing in the journal *Systematic Zoology*, each year since its inception in 1952. These papers represent applications of each research programme to real-world problems.

If we look at the first graph (Fig. 1.4), we see that when *Systematic Zoology* first appeared, most applied papers used the techniques of synthetic systematics. In about 1965, phenetic applications began to appear, but it is apparent that synthetic applications never died out. In the early 70s, phylogenetic systematics began to become popular. At just about that time, just as phenetics was ebbing, and phylogenetic systematics was rising, there was an upsurge in synthetic systematics, peaking in 1974. Right now, we are in the phylogenetic phase of the graph, but neither pheneticist nor syntheticist papers have stopped appearing. The second graph (Fig. 1.5) represents the number of philosophical papers published. It is clear that that the peak periods of the graph straddle approximately the periods of systematic revolutions. Compare these graphs with those that plot the consequences of the Received View and Kuhn's models of science (Figs. 1.2 and 1.3, respectively), and it is immediately obvious that it is Kuhn's model which best fits the data.

In conclusion, then, it is my contention that both the history of systematic research and the pattern of systematic applications accord well with Kuhn's model of the development of science.

Figure 1.4 The number of papers published in *Systematic Zoology* from 1952 to 1987 applying the methods of synthetic taxonomy (□), phenetics (■), and phylogenetic taxonomy. The trend in applications shows that the use of phenetic and synthetic methods has never been completely replaced by phylogenetic systematics.

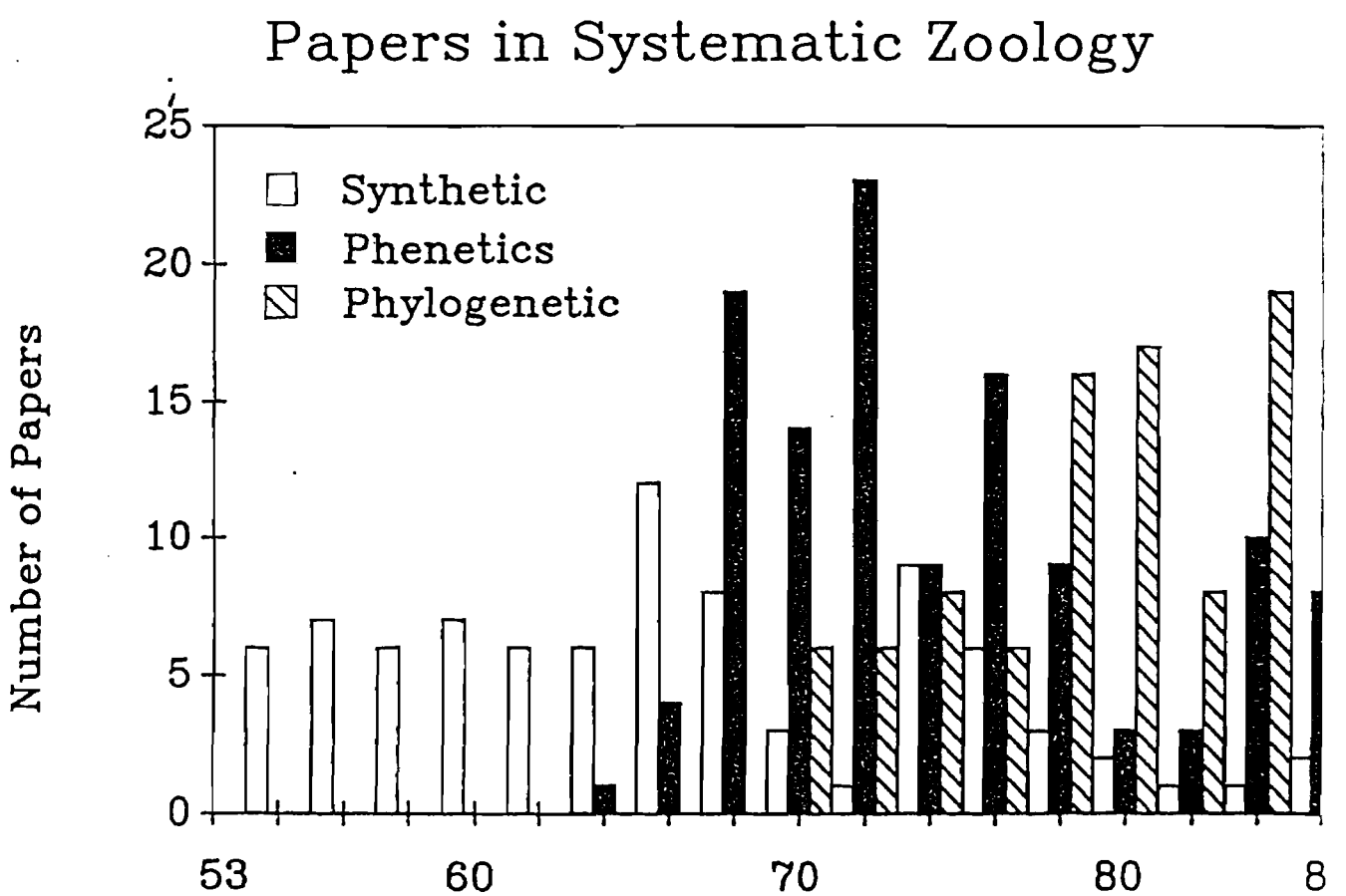
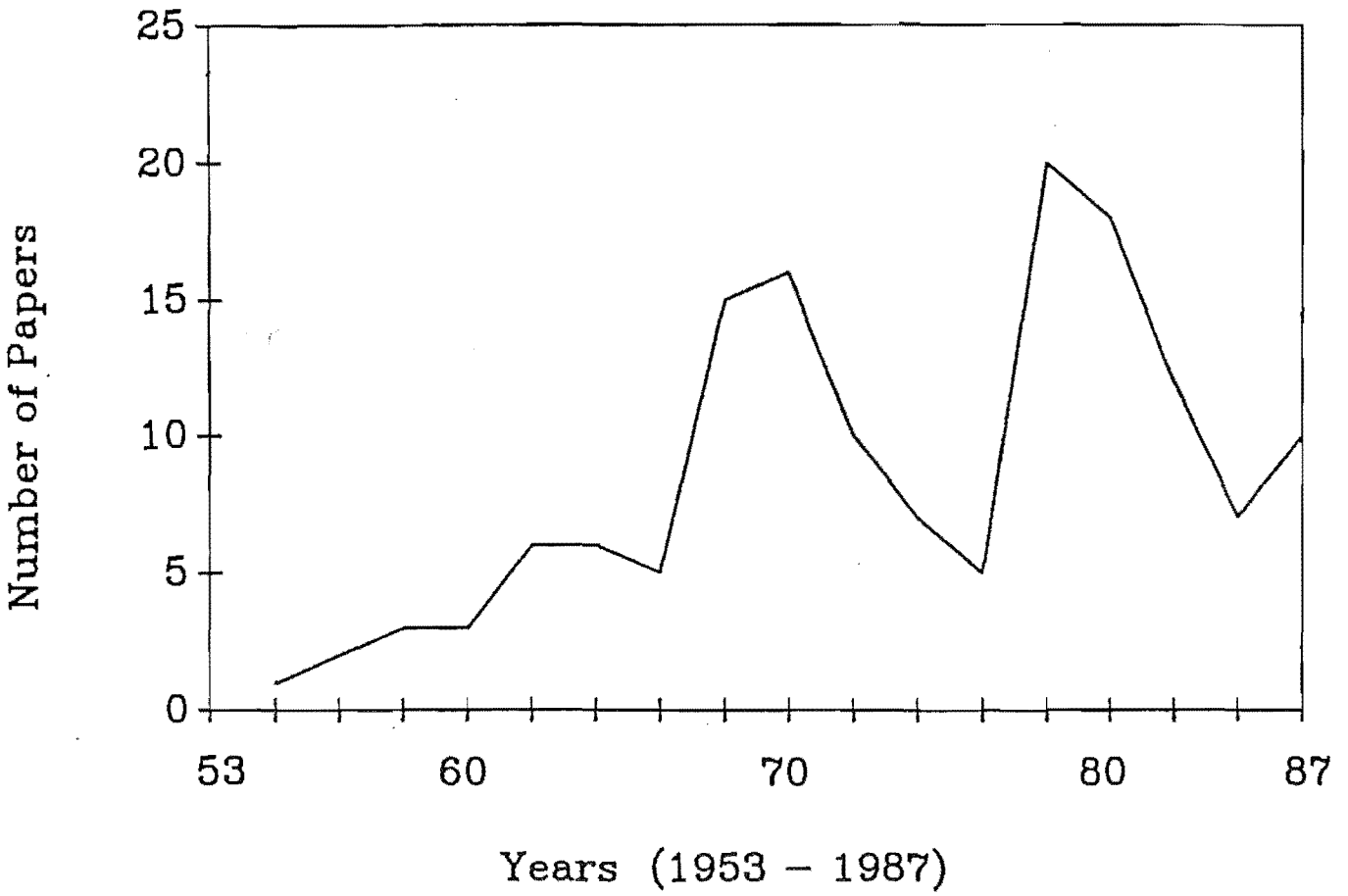


Figure 1.5 The number of papers dealing with concepts and appropriateness of systematic methods, published in *Systematic Zoology* between 1952 and 1987. The peak periods straddle the periods of change shown in Fig. 1.4, as predicted by the Kuhnian model of science.



FOOTNOTES FOR CHAPTER 1

1. The term *paradigm* has come to be used to denote propositions of a research programme which are held to be well-established. However, Kuhn often speaks of working under the Newtonian paradigm, and apparently, he takes this to mean the Newtonian research programme which is the totality of all propositions of Newtonian physics. This vague and imprecise use of the term paradigm has troubled Kuhn and has been provided ammunition for his critics. However, it does not obscure Kuhn's central thesis: there are certain theories, methods and research programmes which are adopted by a community for reasons which are not necessarily empirical.

2. The term *synthetic systematics* was first coined by Farris (1979) to denote the research programme which is now more commonly called evolutionary taxonomy. This latter term is to broad, for phylogenetic systematics also gives due regard to the evolutionary paradigm.

3. To see that this is true, consider the following quote from Mayr (1942):

"The known number in which the above species definition [i.e., the Biological Species Concept] may be inapplicable is very small, and there seems to be no reason at the present time for 'watering' down our species definition to include these exceptions."

4. Simpson (1961) defines the evolutionary species as follows:

"An evolutionary species is a lineage (an ancestral-descendent sequence of populations) evolving separately with its own unitary evolutionary role and tendencies".

5. The self-defining species is a loosely defined concept which relies on the application of the Mate-Recognition Criterion as a means of assessing species boundaries. An oversimplified but essentially correct statement of this criterion is that it assumes that if two individuals recognise each other as potential mates, then they should be treated as members of the same species. Proponents of the Mate-Recognition Criterion see mate-recognition as an instance of species homoeostatic control.

6. More recently techniques which are explicitly statistical have been developed.

ADDENDA

A1.1. A brief review of different systematic techniques

The following is a more detailed review of the main "schools" of systematic thought than the one given in the paper presented at the Arthur Prior Memorial Conference.

Evolutionary systematics. According to Mayr (1969), and Simpson (1961), taxonomy should reflect evolution, taxonomic relationships should be based on shared ancestry, and classifications should be translations of genealogical hierarchies. However, classifications should also contain information about overall similarity. For example, of three Operational Taxonomic Units (OTUs), *A*, *B*, and *C*, if *A* and *B* share a common ancestor not shared by *C*, but *B* and *C* have greater phenotypic similarity, then it is acceptable to classify *B* and *C* as sub-taxa of the same taxon, while placing *A* in a separate taxon. (In effect, this highlights the difference between the evolutionary systematists' and cladists' definitions of *monophyly*: the latter hold that *all* taxa derived from a common ancestor must be included in a monophyletic group. By this definition, if *C* is to be included in a taxon containing *B*, then *A* must be included as well).

In practice, the reconstruction of evolutionary history sometimes involves *ad hoc* decisions about whether morphological features have evolved in response to ecological pressures, or whether they were inherited from ancestral taxa. As a result, evolutionary systematists are accused of subjectivity and "authoritarianism".

Phenetics. The phenetic school of systematics arose as a rebellious response to the "subjectivity" of evolutionary classifications. Pheneticists hold that evolutionary history can never be known (Sneath, 1983). The best classifications, therefore, are those which do not theorise about untestable events, but which attempt to identify groups on the basis of shared taxonomic characters (while previously these were restricted to morphological features, the development of accessible molecular techniques led to the use of cytological, protein, and nucleotide sequence data in taxonomy).

Pheneticists believe that all characters should be given equal weight, and that as many characters as can be obtained should be used (Sokal and Sneath, 1963). There are various techniques and algorithms used to arrive at hierarchical schemes as preliminaries to classification construction, and these are are largely mathematical and computationally complex. However, the burden of computation is carried largely by computers.

Phylogenetic systematics. Phylogenetic systematists maintain that the formulation of a model of evolutionary history is an important prerequisite

to the construction of classifications. However, because of methodological differences, phylogenetic systematics can be divided into four systematic sub-disciplines: Hennigian systematics, cladistics, compatibility analysis, and statistical phylogenetics. Each of these will be discussed in turn; the order in which they are discussed reflects the chronological order of their appearance.

Hennigian systematics. The techniques proposed by Hennig (1950) and expounded by Kiriakoff (1959) have formed the basis of all subsequent phylogenetic systematic research programmes. Hennig argued, rightly, that the phylogeny of a group of organisms can only be reconstructed if the direction or polarity of character states are known, i.e., if we know the temporal order in which character states have evolved. States that evolve later are *derived* or *apomorphic* states, in contrast to *plesiomorphic* states that are inherited from ancestral taxa. It follows that plesiomorphic states offer no information about evolutionary history: even taxa that arise high up in the phylogenetic tree may retain plesiomorphic features. However, if two taxa share apomorphic characters, there is every reason to believe that they share a common ancestor. In accordance with this argument, and by considering all polarised characters, we can infer the phylogenetic history of a group of organisms.

Hennig realised that there would be occasions when characters would provide conflicting evidence about phylogenetic relationships. This occurs when two apomorphic character states identify different but overlapping hypothetical monophyletic groups. For example, Character *X* might partition a group of four taxa (*S1*, *S2*, *S3*, and *S4*) into two groups {*S1*, *S2*} and {*S3*, *S4*}. However, Character *Y* may split the four taxa into groups {*S1*, *S3*} and {*S2*, *S4*}. Clearly, *X* and *Y* cannot both be indicators of monophyly. In such circumstances, Hennig advocated using his technique as an exploratory tool to uncover the inconsistencies in the data, and to direct future research to uncover a means of resolving the problem.

Cladistics. Both cladistics and compatibility analysis were developed in order to provide a solution to the problem of inconsistent phylogenetic hypotheses. Farris and Kluge (1969) developed what they called quantitative phyletics, which was in principle, based on the Camin-Sokal method of Minimum-Steps Evolution (Camin and Sokal, 1965). According to this method, the best hypothetical phylogeny is the one that requires the fewest number of character changes across all characters. Thus, a "parsimonious" arrangement of character changes best resolves the inherent inconsistencies of the character-taxon data (hence, the method is usually referred to simply

as "parsimony").

Parsimony has been defended on a number of philosophical grounds, and these will be reviewed later.

Compatibility analysis. Compatibility analysis is based on the reasonable idea that in any group of taxa, there are some characters that have had only one change of state (i.e., there have been no parallelisms, convergences, or reversals for that character in the evolutionary history of that group of taxa). It stands to reason that since there can only be one true phylogenetic tree for any particular group of taxa, all such uniquely derived characters can be arranged on the tree in such a way that there will be no conflict of evidence, i.e., the characters will be compatible.

Compatibility analysis works backwards from this point: if the set of uniquely derived characters can be identified then the phylogenetic tree can be reconstructed. If it is assumed that characters behave independently, then it is unlikely that any large subset (or *clique*) of characters, each of which is compatible with other characters in the clique, could have arisen by chance. It is reasonable, therefore, to assume that such a subset must indicate that the characters have arisen only once, and therefore can be used to reconstruct the evolutionary tree.

There are two problems with compatibility analysis. First, it is highly likely that the largest clique of compatible characters will not completely resolve the tree; and second, characters that are not part of the compatible clique do not contribute to the resolution of the tree (but see Strauch, 1984, for a solution to both of these problems).

Statistical phylogenetics. A statistical approach to phylogenetic reconstruction was the next step in a natural progression from the numerical techniques of phenetics and parsimony. The earliest, and consequently, the most well known of these approaches is maximum-likelihood estimation (Felsenstein, 1973; Farris, 1973; also see Chapter 5). This method attempts to uncover the topology of the phylogenetic tree for which the character-OTU data has the highest probability of being observed.

Another technique which has been developed by Penny, Hendy and their co-workers at Massey University, New Zealand, involves a least squares approximation of topology and rates of character change such that the differences between the observations and the estimates predicted by the tree are minimised (Penny *et al*, MS; D. Penny, pers. comm.).

There are two problems with statistical estimation techniques: first, there is a lack of accessible software with which the requisite computations can be made; second, restrictive assumptions are often made to relieve the

computational burden, and these fail to reflect the complexity of the evolutionary process.

If there are no inconsistencies in the data, Hennigian trees, and those recovered using parsimony, and compatibility analysis are identical (assuming that there are sufficient data to fully resolve a dichotomous topology). Furthermore, if we assume that rates of character change are low and equal, and branch lengths (i.e., times between cladogenetic events) are not too different, then all phylogenetic techniques usually arrive at the same estimate of evolutionary history.

A1.2. A further note on incommensurability

Perhaps the most distressing aspect of Kuhn's thesis of incommensurable paradigms or research programmes is the fact that it makes science seem so arbitrary and irrational. However, I think this is due more to a misunderstanding of the correct nature of incommensurability, than to any suggestion on Kuhn's part that science is indeed an irrational enterprise.

The literal definition of incommensurability was given in a quote at the start of this chapter: the inability to find a common standard by which to measure two or more objects. This definition applies equally well when referring to the incommensurability of research programmes. By what criteria can the relative value of one systematic method be compared with that of another ? Should we measure general applicability, ease-of-use, stability, naturalness (whatever that is), testability, verifiability ? It is almost certain that if all of these are measured (always assuming that different scientists can come to a consensus about how these qualities *can be* measured), there will be no one systematic method that will have the highest score for *all* criteria (despite claims by cladists to the contrary; see Chapter 2). How then can a suitable method be chosen ?

In my opinion, the choice must necessarily be subjective, i.e., a scientist must choose that method which has the "highest score" for the quality he/she values above others. However, it is wrong to confuse subjectivity with irrationality. We say that someone behaves rationally if he/she chooses a course of action (or the *best* course of action) that will result in the fulfilment of a predefined goal (Newton-Smith, 1981:241). A systematist working on a group of organisms has detailed knowledge about the biology of the group, as well as some idea about the utility of his/her classification. For instance, entomologists know that their classifications form the basis for comparative biological research, as well as pest management, and conservation work (Danks, 1988). Hence, the need for

classifications that will satisfy the needs of most biologists, resource managers, and conservators who rely on such classifications as a primary source of biological information. On the other hand, microbiologists and protistologists who work at the level of the molecule and cell may require classifications that reflect *overall similarities* of molecular characteristics, whether these molecules are genes or enzymes. Wayne Moss (1983) states the case elegantly:

> "The fundamental question for a taxonomic revision is whether the adoption of a numerical technique provides insight into the relationships, classification, and phylogeny of the taxon under study. If not, then the method is either inferior or else irrelevant to the taxon."

A systematist therefore makes a decision about which method will accomplish the goals that he/she sets. These goals relate to the functions that the systematist believes a classification should perform. While this decision obviously rests on subjective foundations, it is nonetheless, a rational one.

CHAPTER 2

WHAT IS THE REAL DIFFERENCE BETWEEN NUMERICAL TAXONOMIES ?

A paper presented at the Annual Conference of the Systematics Association of New Zealand, 1988, held in Christchurch, New Zealand

"Perfection of means and confusion of goals seem
- in my opinion - to characterise our age."
Albert Einstein
Out of My Later Years

Lord Kelvin, the British mathematician and physicist said "When you can measure what you are speaking about and express it in numbers, you have in your thoughts advanced to the stage of science". This intimate association between mathematics and scientific enquiry, however, predates the 19th century and has its roots in the philosophy of Rene Descartes (Lossee, 1980:73). Biologists, slow at first to come to terms with numerical methods, have caught up with a vengeance. Today, in all fields of biology there is a heavy reliance on numerical tools - statistics, calculus, and numerical analysis. In systematics, the advent of computing machines provided the perfect environment for NUMERICAL TAXONOMY to flourish. Initially, the term *numerical taxonomy* was used only in reference to phenetic methods i.e., methods that erected classifications on the basis of overall phenotypic similarity (Sokal and Sneath, 1963). However, since the 1980s, it has been applied to all taxonomic methods that rely on numerical procedures for the development of hypotheses of relationships, or classifications[1]. The term is used to distinguish these methods from *classical* or *evolutionary taxonomy* which relies heavily on the expertise and subjective interpretation of the taxonomist[2].

I will not discuss the differences between evolutionary and numerical taxonomy. Instead I will focus my discussion on the methods of the latter, for it is on these that the spotlight is presently directed.

In the discussion that follows, I will illustrate the main points of my argument with examples drawn from the following numerical taxonomic methods:

a) *Parsimony*, in which classifications are constructed on the basis of the simplest hypothesis of phylogenetic relationships;

b) *Compatibility*, in which classifications are constructed on the basis of phylogenetic relationships which are supported by the largest number of taxonomic characters;

c) *Phenetics* in which classifications are constructed on the basis of overall similarity; and finally

d) *Statistical methods* (in particular, maximum-likelihood estimation), in which classifications are constructed on the basis of the most likely evolutionary tree.

Before discussing the differences between these taxonomies, let us examine the common ground[3]. As systematists, we all accept:

First, that *taxonomy is a service science*[4]. The classifications which we erect are used by biologists for comparative research, by resource managers as databases of biological information, and by conservators for wildlife

management (Mayr, 1982:239; Abbott *et al*, 1985).

Second, that *hypotheses of relationships are rarely testable in practice*[5]. This holds true whether the classification is a phylogenetic one or a phenetic one (Hull, 1984:19).

Third, that *information is usually lacking about the quality of the taxonomic characters used to define taxa.* We seldom know, for instance, whether a character is ancestrally determined, or ecologically determined, and rarely can we come to any conclusion about the rate of change of these characters.

While proponents of different numerical taxonomies agree on the validity of these fundamental precepts, they rarely agree about anything else. Why should this be so? In this paper, I will present a partial explanation for this difference of opinion. I will argue that:

1. The different "schools of taxonomic thought" correspond to different scientific research programmes each defined by its own set of goals and methods. The differences between numerical taxonomies are in fact differences in the paradigms of these research programmes.

2. Criticisms of different research programmes must ultimately be directed at the paradigms of these programmes. This is difficult because the fundamental premises of different research programmes are uninterpretable in the light of paradigms of other research programmes; and finally

3. Even between research programmes that share the same goals, there may be differences in approaches to attaining these goals. These approaches fall into two broad categories depending whether *methodological tools* or *modelling procedures* are used.

Before I proceed to defend these theses, however, it is necessary to review the nature of scientific research programmes, and their properties. A research programme, is, as the name suggests, a programme of scientific enquiry, conducted by a community of like-minded scientists who share the same ideas *vis-a-vis* methodology, established theory, "scientific facts", and observation (Lakatos, 1978). These shared beliefs are referred to as *paradigms.* It is important to recognise that paradigms make up the relatively unassailable "hard core" of scientific research programmes. Our unquestioning acceptance of the paradigms of our research programmes provides us with the foundations upon which we may build our theories.

Consider, for instance, the research programme that is evolutionary biology. One of the major paradigms of evolutionary biology is that acquired somatic adaptation cannot be passed through the germline to progeny (Mayr, 1982:701). In all evolutionary models, this thesis is taken as

read. No referee nowadays ever rejects a paper because the author fails to consider the inheritance of acquired characteristics. And attempts to overturn this paradigm has been met with cynicism, derision, and even hostility (for example, see Mitchison's (1980) review of Steele (1980)).

As taxonomists, we too have our paradigms: we accept, for instance, that existing species have arisen from preexisting ones. We accept that the characters recognised in our study organisms convey information about their lineage. These paradigms of our research programme go unquestioned, unassailed.

One final word on the naturé of paradigms: it was a historian of science, Thomas Kuhn, who suggested that the paradigms of different research programmes are often *incommensurable* - that is, the paradigms of one research programme cannot be interpreted completely in terms of those of another research programme (Kuhn, 1970). As a result, theories which arise as a consequence of the paradigms of one research programme, may turn out to be completely uninterpretable when scrutinised within the framework of another. We have a splendid example of this in systematics, a debate which has been raging for 200 years: the definition of a species. Why are there so many species concepts ? The reason, I am certain, lies in the fact that different systematists build their definitions on different paradigms. Contrast, for instance, Mayr's (1942) *Biological Species Concept*, with the *Self-defining Species* of Lambert et al (1987). Both groups claim that their "species" have a real existence in nature and yet their concepts are in diametric opposition. This difference in opinion can only be explained by the fact that both groups have a different concept of "biological reality". And this is, in fact, the case. Lambert and his co-workers are structuralists: they view organisms and environments as intimately-linked supersystems (Hughes and Lambert, 1984). Mayr, on the other hand, is a Neo-Darwinian, and his species have an organic integrity quite divorced from the environment (Mayr, 1970:39). Neo-Darwinians have difficulty understanding the structuralists and the converse is equally true.

How can all this help us understand the differences between numerical taxonomies? First, I think it is important to realise that numerical taxonomies are not just methods for erecting classifications, in the same sense that, say, light and electron microscopy are methods for examining structure. If that were the case, then the controversy would be easily resolved. Instead, numerical taxonomies are classification systems that have grown out of different research programmes, each with its own constellation of paradigms (Hull, 1988).

To see that this is true, we need only consider some of the paradigms of any two different numerical taxonomies. For example, consider *compatibility analysis* and *phenetic taxonomy*. A fundamental premise of compatibility analysis is that taxonomic data contain phylogenetically-relevant information, and that classifications should reflect phylogeny. The method advocated by proponents of compatibility is simply an extension of a philosophically sound principle: the best hypothesis is the one supported by the greatest amount of information (Meacham, 1984; also see Addendum A1.1).

Contrast these premises with those of phenetic taxonomy. The major premise of phenetics - that taxonomic data contain "biologically-meaningful" information - is more general than the equivalent paradigm in compatibility analysis. Secondly, pheneticists believe that classifications should be informative rather than phylogenetic; and finally, pheneticists argue that the most similar taxa share more biologically-meaningful information than those which are less similar. Therefore, from this argument, it follows that the best classifications should reflect overall similarity (Sneath and Sokal, 1973).

Proponents of both systematic research programmes (compatibility and phenetics) disagree fundamentally in their interpretations of the nature of taxonomic data, the role of classifications, and appropriate methodology. Can we ever hope to reconcile these differences? I think not. For instance, one of the criteria often used to compare different classifications is *naturalness*. Cladists claim that parsimony methods provide natural classifications because these methods identify "real" hierarchies of taxa - in other words, parsimony reconstructs the evolutionary tree (Wiley, 1981). Pheneticists, on the other hand, claim that their classifications are natural because they reflect the order and organisation of properties of living organisms -to them, classifications are natural in the same way that Mendeleef's Periodic Table is natural (Rosenberg, 1985).

I maintain that we can never hope to resolve the conflict between taxonomic research programmes because the paradigms of these programmes are incommensurable. The very criteria by which we attempt to judge the merits of these programmes - *naturalness, information-content, predictiveness* - are interpreted differently by pheneticists and cladists. Only someone not allied to any research programme could conceivably provide an objective interpretation of these concepts. It is unlikely that such a person can be found, because ultimately the interpretation of the concepts mentioned above fall within the purview of the philosophers of science, and even they

cannot agree on the correct interpretation!

I submit, therefore, that any argument which criticises an alternative taxonomic research programme method should be evaluated with the following consideration in mind:

Is it likely that the underlying paradigms of the two research programmes are incommensurable ?

Let us now turn our attention to evaluating numerical taxonomic research programmes which share the same goals *vis-a-vis* the kind of information a classification should preserve. Superficially, it would seem that if taxonomists are united by a common goal, then any differences that exists (say, in methodology) are easily reconcilable. Unfortunately, this is not necessarily true.

As stated earlier, there are two broad classes of taxonomic methods currently in use: *methodological tools* on the one hand, and *modelling procedures* or *model systems* on the other. Briefly, the difference between these two kinds of methods rests on the nature of their underlying assumptions. Methodological tools are taxonomic procedures which rely on scientifically established or philosophically sound methods for the generation of hypothetical relationships (Fig 2.1). In other words, these hypotheses are a direct consequence of the paradigms of a research programme. Modelling procedures, on the other hand, rely not only on the paradigms of the research programme, but, also on a set of empirical assumptions which may or may not hold true (Fig 2.2). The result is the same: a classification is generated, and together with the empirical assumptions make up a model (see Harre (1986) for a discussion of the properties of models).

By this definition, cladistics, which invokes the principle of parsimony, is a methodological tool, whereas maximum-likelihood estimation, a statistical technique, is a modelling procedure. In fact, many modelling procedures are statistical methods. The reason for this is obvious: many statistical techniques make testable assumptions about the nature of the data (for instance, assumptions about distributions, and independence).

In order to compare modelling procedures with methodological tools I will introduce the notion of justifiability:

The use of a technique is justifiable, if and only if, it is scientifically defensible, and the underlying assumptions are not known to be false.

This definition corresponds intuitively with the common usage of the term "justifiable", and if one accepts this criterion, then it is clear that the use of methodological tools is almost always justifiable. This is because the methods are based on accepted principles, i.e., the assumptions of the

Figure 2.1 Methodological Tools. The central "hard core" of a methodological tool consists of paradigmatic assumptions, goals, and methods. On the basis of these paradigms, hypotheses are erected for any given data set.

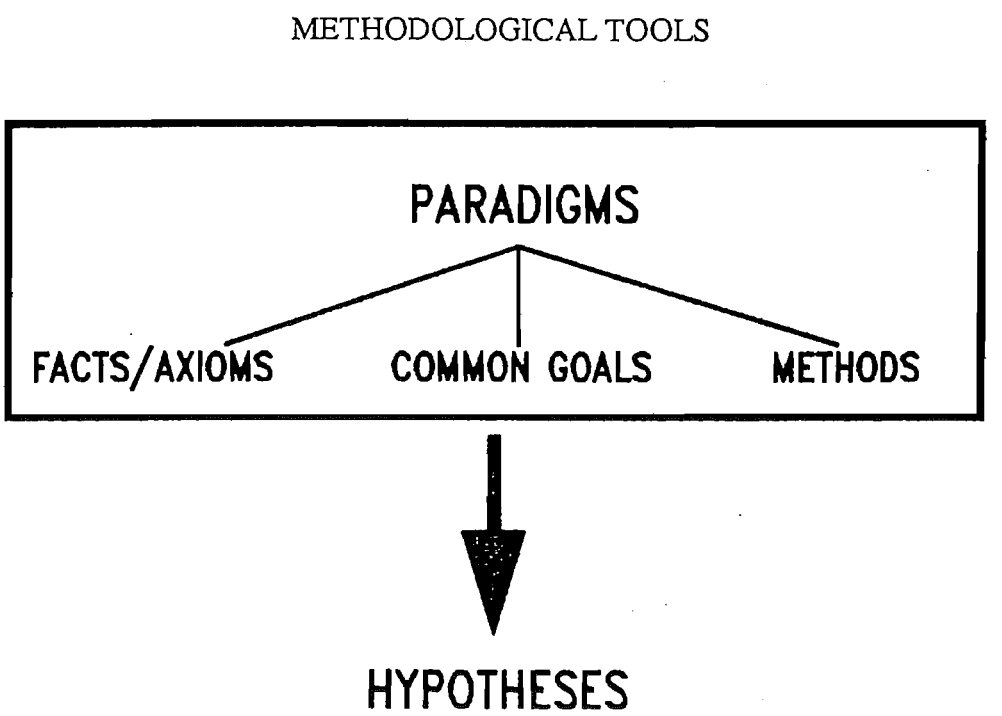
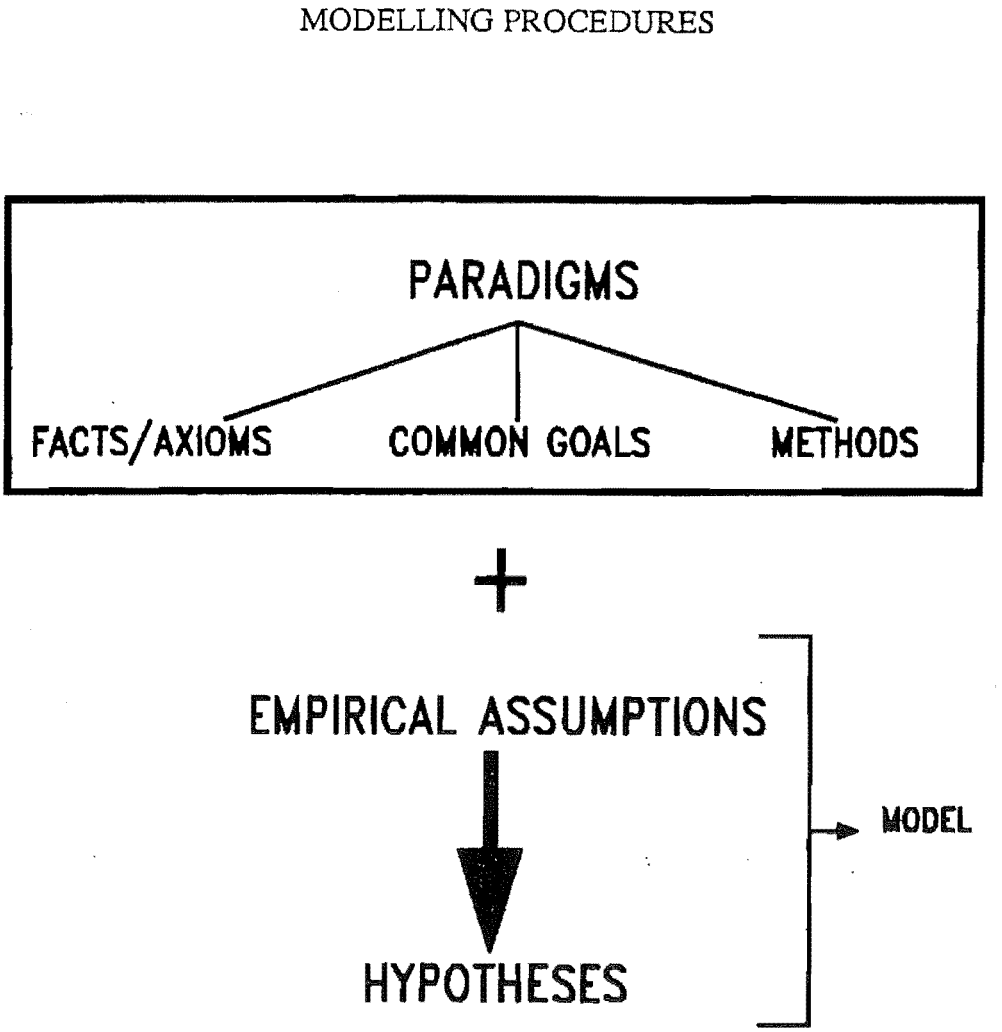


Figure 2.2 Modelling Procedures. Modelling procedures have both a "hard core" of paradigms, and a "mantle" of empirical assumptions. Hypotheses erected using modelling procedures are susceptible to falsification if it can be shown that the empirical assumptions on which these procedures are based are false.



methods are themselves paradigms. Take, for example, cladistics. If we accept the paradigms of the cladists' research programme, it will always make sense to use parsimony methods to construct classifications [Note: Kluge (1984), for instance, defends parsimony strictly on the basis of methodological principles citing Popper and his philosophy as support].

How does the criterion of justifiability apply to model systems? The use of model systems is *not always* justifiable. This may be because the modelling procedure is not defensible; but more often than not, it is because the assumptions on which the model is based are inappropriate.

A good example of this is the maximum-likelihood estimation of evolutionary trees[6] . An examination of the basic premises for maximum-likelihood estimation reveals a number of weak points: these revolve around the appropriateness of the empirical assumptions (see Addendum A1.1 and Chapter 5). For instance, we would question the validity of assuming that characters evolve independently, and certainly, we have no reason to believe that the time intervals between speciation events are equal.

If we operate within a phylogenetic framework, and we agree that classifications should represent phylogeny, then the decision to choose a methodological tool, such as parsimony, or a modelling procedure, such as maximum-likelihood estimation, depends, to some extent, on what we know, or can infer, about the biology of the taxa in question and the evolutionary process, in general. It is my opinion, however, that modelling systems, have one quality that methodological tools lack: the hypotheses erected by the former are more sensitive to empirical data. By this I mean that it is easier to falsify a taxonomic hypothesis erected using a modelling procedure than one derived by some application of a methodological rule. In the case of the former, we can attack the hypothesis head on, or we can attack the underlying assumptions of the model. The underlying assumptions of methodological tools, however, are more impervious to attack. This difference is, I believe, important enough to tip the scales in favour of using modelling procedures.

FOOTNOTES FOR CHAPTER 2

1. The volume *Numerical Taxonomy* edited by Felsenstein (1983), for instance, has papers on cladistics and compatibility.

2. See Addendum 1.1.

3. When I started this paper, I was convinced that some common ground could be found. However, in discussions with systematists, I began to realise that there was not a single statement which was accepted without qualification by all.

4. That taxonomy is a *service* science is certainly controversial. When I mentioned this to a colleague he replied that he did not think it necessary to relegate systematics to the position of subservience amongst the biological sciences.

5. Pheneticists maintain that relationships based on overall similarity are testable. However, they maintain that these relationships can be tested by accumulating evidence from different data sets, i.e., testability by verification. However, cladists find testability by falsifiability the only true test of a theory. Therefore, there is no way by which hypotheses of relationships can be *objectively* tested.

6. Examples of constraints of the Maximum-Likelihood model given in this paper correspond to the model derived in Chapter 5.

ADDENDA

The conference paper was, by necessity, brief. In this section, I clarify some of the points that were glossed over. Since my main concern is with phylogenetic systematics, and because cladistics is now seen as its mainstream technique, I discuss the philosophical implications of cladistic methodology.

A2.1. A note on justifiability

Classifications represent hypotheses of relationships. I think there can be no disagreement about that. There are differences, however, in what systematists believe the nature of these relationships should be. For instance, pheneticists believe that systematic relationships should be based on overall phenotypic similarity, whereas phylogeneticists (and evolutionary taxonomists, for that matter) hold that classifications should reflect genealogical relationships.

Whichever position one holds, systematic methods are designed to uncover the most satisfactory hypotheses about OTU relationships. There is, therefore, a correspondence between the justifiable use or selection of a particular systematic technique, and the acceptability of a systematic hypothesis:

A technique is justifiable if it leads to an acceptable hypothesis of relationship.

Acceptability of an hypothesis differs with different research programmes. Pheneticists, in attempting to estimate the population classification from a sample of characters (Sneath, 1983), obviously place a high value on stability and verifiability. Cladists, on the other hand, believe that only the simplest (most parsimonious) hypotheses are "scientifically acceptable". Acceptability, therefore, can only be assessed from within a research programme. Pheneticists, for instance, believe that inductive techniques can generate "scientifically acceptable" hypotheses. Cladists, however, use parsimony because "only parsimonious hypotheses can be defended by the investigator without resorting to authoritarianism or apriorism" (Wiley, 1975:236). The implicit ambiguity of the definition of justifiability in no way weakens it; rather, it emphasises the importance of the phenomenon of incommensurable paradigms.

Also, in the preceding discussion, reference is made to the "underlying assumptions" of taxonomic procedures. What are some of these assumptions?

Pheneticists assume that character-taxon information gives a clue to the nature of the "population classification", a term Sneath (1983) uses to denote the parametric classification. (Sneath views the construction of classifications

in the same light as, say, statistical regression. In both cases, an attempt is made to identify a parametric or populational trend, from a sample of values). Cladists, on the other hand, assume that character-taxon information is phylogenetically relevant, and can be used to construct a phylogenetic tree. Proponents of maximum-likelihood (ML) methods go one step further, and make assumptions about the rate of change of these characters, and the times different lineages have taken to evolve (branch lengths).

A2.2 Methodological Tools versus Modelling Procedures

The assumptions mentioned above, more than any others, highlight the differences between what I have termed *methodological tools* and *modelling procedures or systems.* Consider, for example, cladistics (as a methodological tool) and ML estimation (as a modelling procedure). Both are used to erect phylogenetic hypotheses. However, the former technique is based on a paradigmatic assumption (i.e., that characters contain phylogenetic information). Add to this the intuitive appeal of Occam's Razor (and its philosophical implications), and *all* hypotheses that cladists erect are satisfactory ones (always keeping in mind that satisfactoriness is assessed from within a research programme).

In order to show that a most-parsimonious tree is an unacceptable hypothesis (and therefore that use of the method is unjustifiable), one would either have to show that characters have no phylogenetic information, or that Occam's Razor does not have the philosophical endorsement that cladists claim. This would be difficult to do on both counts. Except in rare instances, most biologists accept that characters do carry some phylogenetic information; and the application of parsimony has a strong basis in the philosophy of science. While we can show that most-parsimonious estimates of phylogenetic trees are subject to all kinds of errors (Felsenstein, 1978; Hendy and Penny, MS) cladists maintain that the philosophical benefits of parsimony as an hypothesis-generating procedure far outweigh any inaccuracy in phylogenetic estimation. Farris's (1983) aggressive defense of parsimony is, perhaps, the best example of this (the italics are mine):

> "It seems that no degree of abundance of homoplasy is by itself sufficient to defend choice of a less parsimonious genealogy over a more parsimonious one. That abundance can diminish only the strength of preference for the parsimonious arrangement; *it can never shift that preference to a different scheme.*"

A particular application of ML estimation, on the other hand, can be

rejected as unjustifiable if it can be shown that, for a given data set, the empirical assumptions of rates of character change, etc., are wrong. Modelling procedures are more amenable to empirical evaluation than methodological tools.

As illustrated in Figs. 2.1 and 2.2, one of the characteristics of a research programme is the use of a common method or technique. If the technique in question is a methodological tool, then any attempt to show that the use of that technique is unjustifiable is a near-insurmountable task, because it involves overturning paradigms, both empirical and philosophical. The justifiability of modelling procedures, however, are far easier to contest: all one needs to do is show that the empirical assumptions of the model are unfounded.

A2.3 A critique of Cladistics

I have argued that cladistic methodology, which involves the use of parsimony to construct phylogenetic hypotheses, cannot easily be criticised. Any criticism must be levelled against the paradigms of the cladistic research programme. In this section, I examine the cladists' assertion that the use of parsimony results in satisfactory phylogenetic hypotheses.

A number of different statements have been made by cladists about what constitutes a satisfactory hypothesis. Kluge (1983:31) neatly states the cladist's position, and places the stamp of authority on it:

> "According to Popper (1968:144-145), choosing the most parsimonious, the simplest, hypothesis is more than a way of avoiding a dead end. 'It is a direct corollary of the falsification criterion' for hypothesis testing (Gaffney, 1979:98)."

While Farris (1983:36) agrees that the use of parsimony follows naturally from the Popperian philosophy, he believes that parsimony can also be defended on other grounds:

> "...in applying the parsimony criterion, [phylogenetic analysis] chooses among alternative hypotheses of relationship on nothing other than their explanatory power"

where explanations are

> "...judged on their ability to cover observations with few boundary conditions, that is, with little extrinsic information....The explanatory power of a genealogy is consequently measured by the degree to which it can

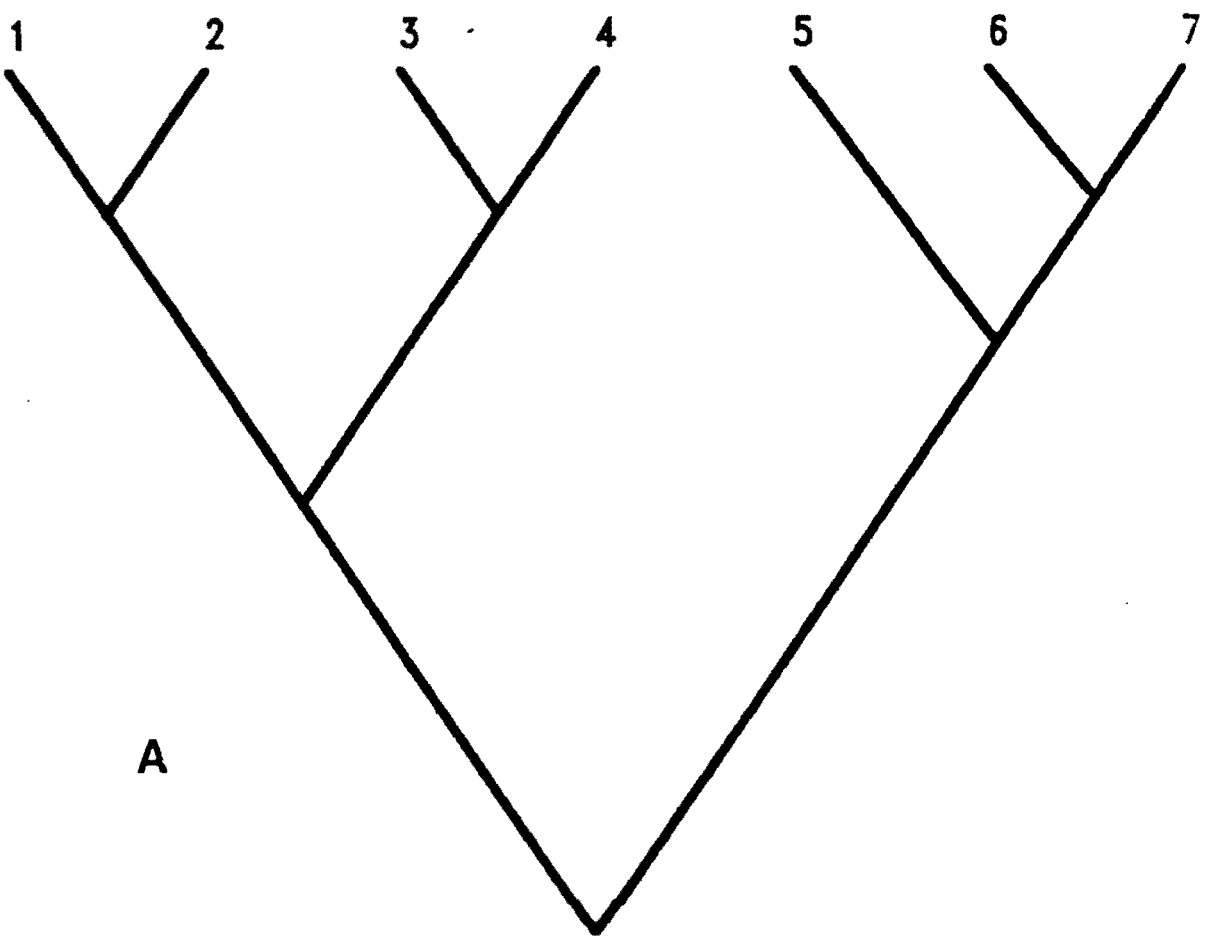
avoid postulating homoplasies."

Finally, in attempting to fight the opposition on their own turf, some cladists have attempted to show that the very things pheneticists claim their methods do well are better done by using cladistic techniques. For instance, Farris (1979, 1980) has argued that classifications based on parsimonious cladograms are more informative, more efficient, and more predictive than those based on phenetic dendrograms. Mikevich (1978) has challenged the pheneticists' claim that their classifications are more stable; instead, her results suggest that cladistic techniques lead to more stable classifications. Schuh and Polhemus (1980) have also tried to show that, despite what pheneticists say, there is greater agreement between cladograms constructed using different character-sets of the same OTUs, than between dendrograms.

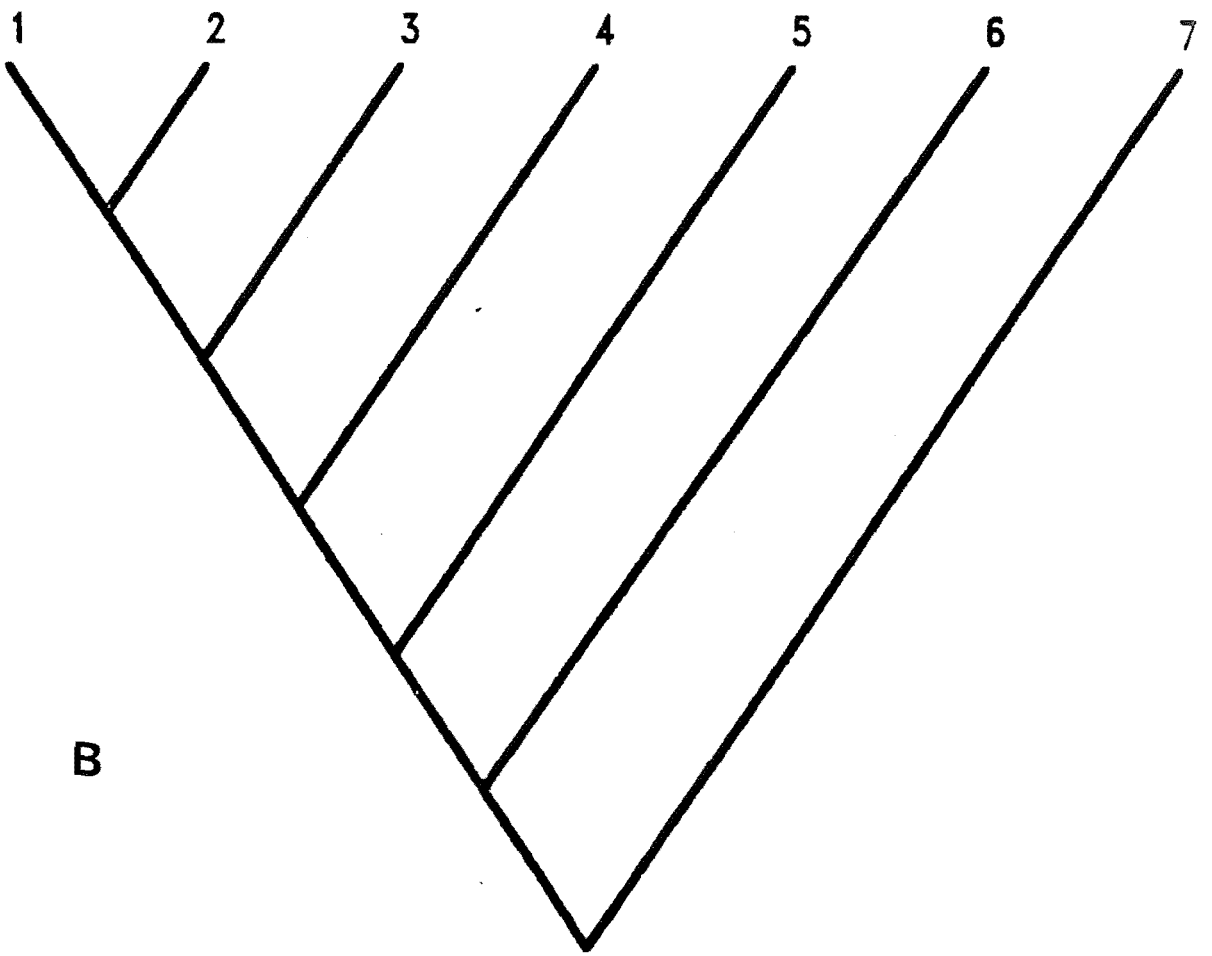
This last set of "justifications" for the use of parsimony are amenable to empirical evaluation. Such evaluations have indeed been undertaken by several workers, the results to date have been equivocal. Studies by pheneticists, for instance, have shown that both phenetics and cladistics perform well with respect to stability and congruence under different circumstances (Sokal, 1983; Rohlf *et al*, 1983). Obviously, workers on both sides stand the risk of being accused of bias, so the question will remain unresolved until methods for testing classifications can be agreed upon.

Farris's (1980) comments on the efficiency and informativeness of cladistic classifications are also open to criticism. He claims that cladistic classifications can be constructed with the fewest number of diagnostic statements, and still be able to retrieve all the character information available (this is his definition of diagnostic efficiency and information content). It is a moot point as to whether the *number* of diagnostic statements a taxonomist needs to make is of great consequence, but even if it is, Farris's argument is still flawed. This is because he implicitly assumes that there is only one way to construct a classification from a cladogram (or any branching diagram): the method of subordination (Cracraft, 1974). By this method, each level of a cladogram translates to a new rank in a classification (Fig. 2.3 illustrates the application of this method). However, although the method of subordination allows a systematist to recover the topology of the cladogram completely, it can be very impractical, particularly when the topology of the tree becomes more asymmetric (e.g., the tree in Fig 2.3b requires more ranks than that in Fig 2.3a). An alternative, and more practical, solution is the "phyletic sequencing method" (Fig 2.4). By this method we can recover the structure of the tree only by reviewing the *order* in which the classification is written. It is a

Figure 2.3 Translation of a phylogenetic tree into a classification by the method of subordination. Each subordinate clade is accorded a new rank. (A) Symmetric trees require fewer categories than (B) assymetric trees.



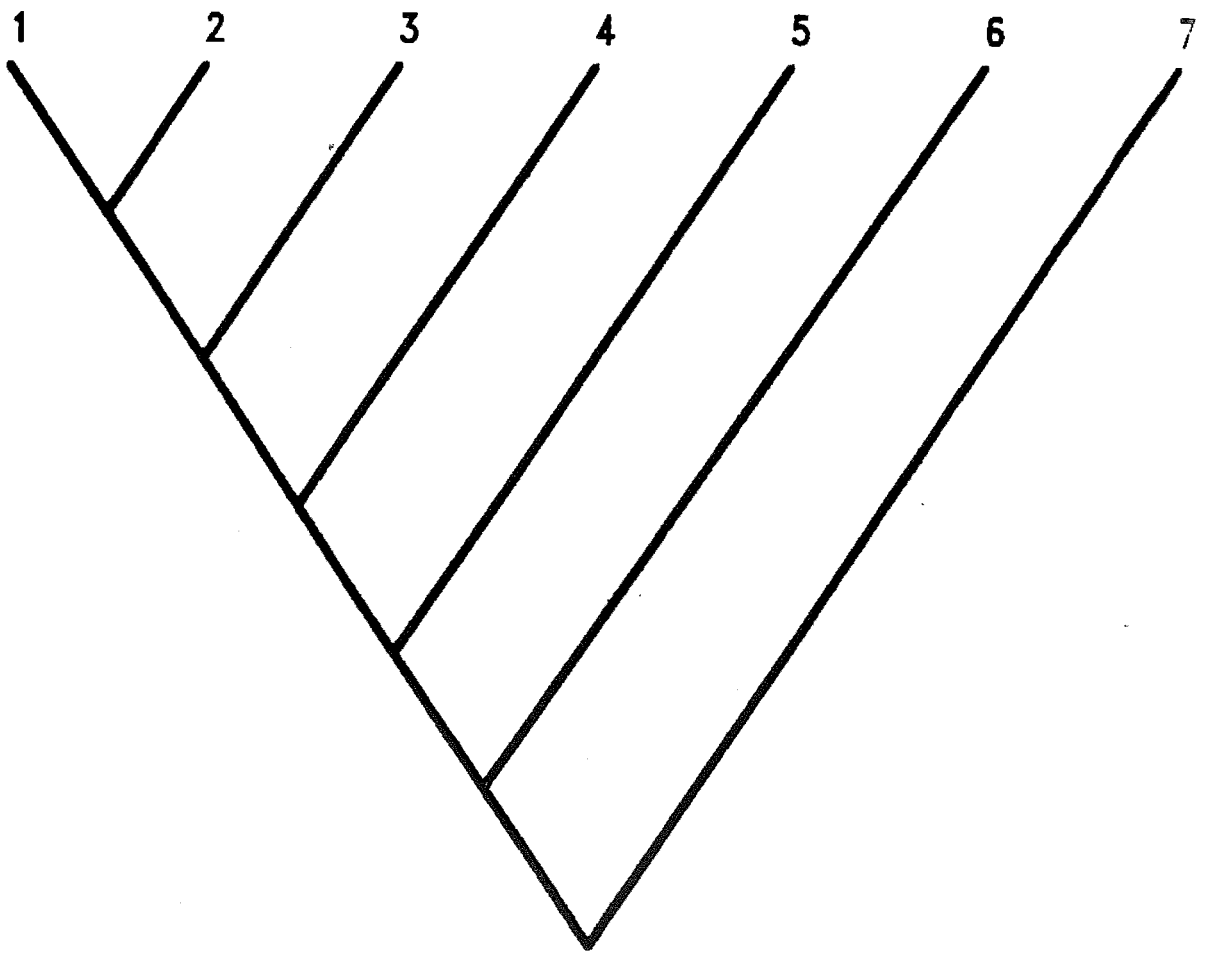
Family (1,2,3,4,5,6,7)
  Genus (1,2,3,4)
    Species (1,2)
      Sub-species (1)
      Sub-species (2)
    Species (3,4)
      Sub-species (3)
      Sub-species (4)
  Genus (5,6,7)
    Species (5)
    Species (6,7)
      Sub-species (6)
      Sub-species (7)



B

Sub-order (7,6,5,4,3,2,1)
Family (7)
Family (6,5,4,3,2,1)
Sub-family (6)
Sub-family (5,4,3,2,1)
Genus (5)
Genus (4,3,2,1)
Sub-genus (4)
Sub-genus (3,2,1)
Species (3)
Species (2,1)
Sub-species (2)
Sub-species (1)

Figure 2.4 Translation of a phylogenetic tree into a classification by the method of phyletic sequencing. Each subordinate clade is given the same rank as other clades if it is derived from the same "trunk" or "branch" of the tree. The number of categories is always equal to or less than that required by the subordination method.



Species (7,6,5,4,3,2,1)
Sub-species (7)
Sub-species (6)
Sub-species (5)
Sub-species (4)
Sub-species (3)
Sub-species (2)
Sub-species (1)

compromise devised to keep the number of ranks to a manageable level. The problem with this method, however, is that it loses the quality that is claimed for cladistic classifications by Farris: classifications erected using the sequencing method *do not* require the fewest diagnostic statements to fully recover character-taxon data, particularly when dealing with asymmetric cladograms.

Nevertheless, the fact that cladistic techniques do not necessarily meet the empirical expectations of its proponents is only a secondary problem. After all, it is the philosophical base on which cladistics rests that decides the question of its justifiable use and cladists are adamant that this philosophical base is unassailable. However, I will argue that their foundation stone is not the bedrock they suppose it to be.

Despite the fact that cladists claim that parsimony is a direct application of Popperian philosophy (Wiley, 1981), their interpretation of Popper and his *criterion of demarcation* is peculiar. Popper's criterion of demarcation is based on a principle of falsifiability and was originally proposed as a means to separate science from non-science: a theory or conjecture is scientific if one can (potentially) obtain independent evidence that it may be false (Popper, 1974). However, Popper also extends the application of the falsifiability criterion to develop a scientific methodology, and characterise scientific explanations.

According to Popper, an appropriate scientific methodology, insofar as the selection of hypotheses is concerned, would be to choose that hypothesis which has the highest number of falsifiable statements (Popper refers to the number of such statements as the information content of an hypothesis). When there are rival hypotheses with the same information content, Popper suggests that we accept, *for the time being*, the one that has passed the highest number of empirical tests (Popper, 1934). By this means, it is Popper's contention that our scientific knowledge will grow:

> "...every worthwhile new theory raises new problems; problems of reconciliation, problems of how to conduct new and previously unthought-of *observational tests*"
>
> Popper, 1963 (the italics are mine)

Popper places a great deal of emphasis on the *empirical* testing of theories: the more a theory is amenable to observational tests, the stronger it is. Whereas falsifiability is based on a *logical* principle, it is first and foremost an empirical criterion.

Popper uses the same reasoning to decide what constitutes a good explanation (Popper, 1972, 1983). Obviously, the phenomenon to be

explained (the *explicandum*) must follow logically from the set of explanatory statements (the *explicans*). However, to qualify as a good *scientific* explanation, the explicans must satisfy certain additional criteria: they should be true, or at least, not known to be false; if they are theoretical statements, they should be falsifiable; and finally, the number of explanatory *ad hoc* statements should be minimised. The term "*ad hoc*" features a great deal in cladistic philosophy, and it is enlightening to see how Popper uses it. An *ad hoc* statement is one that cannot be tested independently. Non-repeatable events and tautologous statements are examples.

I contend that if we interpret Popper correctly, then cladistics as a method and as a means of explanation fails to meet his standards. [Note: a research programme is, as Hull (1988) points out, very much like a species: among individual scientists, there may be different opinions (=polymorphisms), different problems to solve (=ecological differences), and different strategies to solve these problems (=structural differences). For this reason, we cannot expect to completely identify or characterise either research programmes or species by sets of unchanging descriptors. The very best we can do is describe the variation, and perhaps the more dominant characteristics of the programme, at any one time. What I say here, therefore, does not, and cannot, apply to all cladists. Instead, it represents what I believe are the major theses of the cladistic research programme, as it is today. In particular, I use Wiley (1981), Farris (1983), Kluge (1984), and Brooks *et al* (1984) as sources for cladistic principles].

First, cladists claim that the method of parsimony is a direct consequence of applying the falsifiability criterion. The argument takes the following form: each character represents an hypothesis of monophyly in the sense that groups which share the same derived character state are hypothesised as having "obtained" that character state from a common ancestor (note that this is equivalent to saying that characters represent hypotheses of homologies). Not all characters partition the OTUs in the same way, and some groupings may be inconsistent with others. Inconsistencies among character hypotheses lead to conflicting hypotheses of monophyly. If all characters are considered together on a single tree, then these inconsistencies are taken to be *falsifiers* of a given phylogenetic model in the sense that they represents evidence that contradicts the hypothesis of phylogeny described by the tree, *and* the assumption that every character is an indicator of monophyly. As the cladists see it, then, the best (Popperian) hypothesis is the one that has been falsified the least number of times

(Wiley, 1975).

To accept this, however, is to ignore the implicit and explicit intent of Popper's methodology, i.e., the growth of scientific knowledge through *empirical* testing. Popper certainly advocates the acceptance of a theory that has been tested more often than another, but qualifies this by stating that the theory should have *passed* these tests, not just "passed most often". If a theory is falsified by even one good experiment, then it is not "less false" than if it had been falsified by two, three, or even a hundred experiments [e.g. the Michelson-Morley speed-of-light experiment which provided evidence inconsistent with the theory of an ubiquitous ether (Hawking, 1988)].

Now, it is true, as Farris (1983) remarks, that falsifying evidence may not really be accurate. For example, characters may be recorded wrongly, or what may have appeared to be the same state in two OTUs turns out to be two different states. Hennig (1966), realised this and suggested that inconsistencies of a phylogenetic hypothesis be used to direct systematists to check and recheck their data. By this process of "reciprocal illumination", we may come to know the true phylogeny. But what happens when the inconsistencies remain, even after numerous checks have been made? Does it imply that the proposed model of phylogeny is well and truly false? If it is true that a cladistic reconstruction offers both an hypothesis of phylogeny *and* hypotheses about characters as indicators of monophyly, then the answer must be a resounding "YES".

However, to Popper, the falsity of an hypothesis is not a major worry; what is important is that it stimulates more empirical investigation to resolve the problem. The cladists, however, make a fatal (and, to Popper, unforgiveable) error: they resolve the inconsistency problem by making *ad hoc* statements about what types of evolutionary events may explain the inconsistencies (e.g., by postulating convergent or parallel evolution, and reversals - the inclusive term "homoplasies" are used to describe these events; see Farris, 1983, on *ad hoc* hypotheses in parsimony). The initial assumptions that all characters are equal and good indicators of monophyly are "readjusted", but only for the group of taxa in question. In so doing, cladists are saving their hypotheses by what Popper refers to as "conventionalist strategems", a course of action he particularly abhors (Popper, 1934).

The problem with the kind of *ad hoc* statements cladists make is that they are statements about non-repeatable events that have occurred in the history of one group of taxa. Although it is possible that most-parsimonious hypotheses which incorporate *ad hoc* statements may be true, they lack the

generality that enables empirical tests to be conducted. In other words, they are unfalsifiable.

For instance, a statement of the form "State x of character X arose at times T_1 and T_2 independently in this group of taxa" offers us no way to develop an experiment to show the falsity of this statement. The only way this statement may be falsified is by recourse to the historical record of the group of taxa in question. According to Popper's philosophy, such an hypothesis is uninteresting for two reasons: it tells us nothing about the "behaviour" of Character X in other groups of taxa and their evolutionary histories; and it does not "challenge us to learn; to advance our knowledge; to experiment; and to observe" (Popper, 1972). To reiterate: despite the fact that such a statement may be true, it is a dead end, as scientific hypotheses go.

Now, it can be argued that a cladistic hypothesis about the evolutionary history of a group cannot be deconstructed into separate sub-hypotheses about characters and their particular "evolutionary history". In other words, the only valid test of a cladistic hypothesis is a test of the entire phylogenetic tree, and not of individual characters. However, this is carrying the *holistic* approach to understanding the form of organisms too far. It is evident that morphological features (i.e., potential taxonomic characters) can vary (and thus evolve) independently from other features, at least to some extent. It seems reasonable, therefore, to examine hypotheses about taxonomic characters independently. In short, phylogenetic hypotheses are not simply statements about evolutionary events or relationships, but also about the reliability of taxonomic characters as indicators of these events and relationships.

But even if this was not true, how does one go about testing a phylogenetic tree? Nelson (1978) suggests that a phylogenetic hypothesis may be falsified if a character which is known to accurately describe the phylogeny of a group, disagrees with the hypothesis. Aside from the obvious objection that characters with a known phylogenetic component are few and far between, this statement is true of *any* phylogenetic hypothesis, not just a most-parsimonious hypothesis.

Therefore, whether we treat a cladistic hypothesis as a collection of *ad hoc* hypotheses about evolutionary events (albeit the smallest such collection), or a single hypothesis of evolutionary history, the result is that it is at best as falsifiable as any other phylogenetic hypothesis; at its worst, it violates the principles to which its proponents claim to adhere.

All this still does not solve the problem of inconsistencies and the

ambiguous resolution of phylogenetic hypotheses. Is there any way to propose a model of phylogeny without recourse to *ad hoc* hypothesis ? If not, then perhaps a most-parsimonious phylogeny is the best we can do.

There are two alternatives to dealing with inconsistencies which, in my opinion, are more acceptable by Popper's standards. Essentially, these strategies involve re-evaluating the cladists' central thesis that every character carries cladistic information. For instance, we can make a "weak" modification to this statement, and state that only some characters are good indicators of monophyly. This approach forms the basis of compatibility analysis, which searches for the largest subset of characters that may be used to construct a phylogeny (Meacham, 1980). Other characters are treated as phylogenetically uninformative.

A second, and more general, possibility to the resolution of inconsistencies is to assign to each character a potential rate of change, and construct a phylogeny incorporating these rates; characters with low rates of change will be better indicators of monophyly than those with high rates of change (Felsenstein, 1981). This particular approach can apply to both molecular and morphological data.

Both of these strategies allow us to account for inconsistencies in our phylogenetic hypotheses, but if they all share the same status as conventionalist *ad hoc* methods (i.e., their hypothetical statements are not independently testable), then, by Popper's reckoning, we have not made progress. However, each of the strategies mentioned above add *falsifiable auxilliary hypotheses* to a phylogenetic hypothesis. This is because for both strategies we can make statements of the form, "In general, Character X has a higher (or lower) ancestral component or a lower (or higher) rate of change than Characters Y_1 to Y_n". Statements such as these can certainly be falsified.

Perhaps the best example of this is when nucleotide sequence data is used from non-coding regions of the genome, from pseudogenes, or from mitochondrial DNA. There is good empirical evidence that certain base transitions have a higher probability of change than others (Brown *et al*, 1982). Also, certain mutations such as deletions or insertions of single bases are likely to be far more common than those of longer base sequences (Lloyd and Calder, MS). If we are to reconstruct the evolutionary history of a group of using base sequence data, it makes sense to incorporate what we know about different rates of change into our hypotheses of phylogeny. Indeed, it would be ludicrous not to do so.

For morphological characters, it is more difficult to state and test

hypotheses about relative rates of change. This is because morphological characters co-vary with the ecology of the organism, and with other characters, to different degrees. Any hypothesis about rates of change must take into account both ecological variability and structural co-variation. Nevertheless, it is still possible to specify observational tests that would undermine our confidence in such hypotheses. For example, we may specify that a test of the hypothesis "Character *X* has a higher rate of change than Character *Y*" would be a comparison of the variability of *X* and *Y* in as many taxonomic groups as possible. If the variability of *X* is less than that of *Y*, the hypothesis will be considered falsified (see Chapter 4 for a more detailed discussion).

Auxilliary hypotheses such as these may be specified *a priori* on the basis of initial evidence, or they may be a result of the analysis itself (see Chapter 5). Whatever the case may be, such hypotheses are amenable to falsification on the basis of evidence obtained from taxonomic research on other groups of taxa, studies of intra-population variation, investigation of epigenetic and physiological influence, and biochemical experimentation. In effect, the one property that acceptable auxilliary hypotheses must have to ensure independent empirical testability is general applicability; the higher the number of conditions required for an hypothesis to be tested, the lower the utility and information content of the hypothesis. An *ad hoc* hypothesis has zero information content.

Given that it is possible to propose viable phylogenetic hypotheses by supplementing our character-OTU information with testable auxilliary hypotheses about the nature of our characters, the cladists' justification for minimising the number of *ad hoc* hypotheses as a viable hypothesis-generating method is no justification at all. By the same reasoning, the use of most-parsimonious phylogenies as *explanations* of the observed character distribution of a group of OTUs is equally unjustifiable, by Popper's standards.

One final question remains: is parsimony ever justifiable and useful, and if so, when ? To answer this question, it is important to draw the distinction between most-parsimonious phylogenetic trees with no incosistencies (i.e., phylogenetic hypotheses in which character information provides no conflicting hypotheses of relationships), and trees that have to be resolved by proposing *ad hoc* hypotheses. The former represent hypotheses which are fully consistent with our *a priori* assumptions. Now, it is possible that such hypotheses may be wrong (see Felsenstein, 1978; Penny *et al* MS), but in the absence of any such information, they represent the best

"working hypotheses". As a consequence, the resultant classifications form the basis for novel comparative biological research.

Most-parsimonious phylogenies that incorporate *ad hoc* hypotheses indicate that taxonomic characters used to construct the hypotheses need further evaluation, i.e., such hypotheses point the finger of research at themselves. In such situations, and as Hennig realised, parsimony serves as an exploratory tool. However, as an exploratory tool, it is most effective when only a few *ad hoc* statements are made. This is because as more *ad hoc* statements are added to a most-parsimonious hypothesis, more characters are implicated in homoplasious events. It is not uncommon to find that, for large data sets, *all* characters are hypothesised as having changed more than once (see Archie, 1989). If such an hypothesis is used to direct a re-evaluation of taxonomic information, then it is equivalent to saying, "Re-examine all characters". In such situations, a compatibility analysis, or a consideration of the character rates of change, would be of greater value than cladistic analyses.

In the final analysis, parsimony is certainly a justifiable technique, although it is my opinion that cladists have been misguided in suggesting that it is Popperian. I have come to the conclusion that there is no one all-powerful, all-informative technique. Instead, the best systematic results are achieved by what most systematists know to be essential: good character analysis.

PART II

TECHNIQUES IN PHYLOGENETIC SYSTEMATICS

INTRODUCTION

In the previous section, I outlined the philosophical arguments which led me to re-evluate the view that cladistics offers the best and only acceptable method of phylogenetic analysis. However, as I mentioned earlier, my disenchantment with parsimony arose, first and foremost, because it gave what I felt were unsatisfactory results when used with real data sets. In particular, I encountered two problems:

1. The problem of multiple most-parsimonious phylogenetic trees; and
2. The fact that (unweighted) parsimony analysis does not take into account the relative "value" or "taxonomic merit" of different characters.

The first of these is clearly undesirable from a taxonomic point of view. If the aim of a systematic study is to erect one classification on the basis of a phylogenetic hypothesis, then there can be no room for many equally valid hypotheses.

The second problem is more bothersome. On reviewing character-taxon information for my group of animals, I was sure that some characters were not as "important" as others as indicators of monophyly. How could such information be intergrated into a cladistic analysis ?

A third problem emerged as a consequence of trying to find solutions to the first two. Over the last two decades, phylogeneticists have been pursuing two divergent strategies for evolutionary reconstruction: methodological procedures (e.g., parsimony and compatibility analyses), and statistical techniques (e.g., maximum-likelihood estimation). While it is relatively easy to obtain computer software to perform parsimony or compatibility, statistical estimation programs are not so readily available. This, together with the high mathematical content of papers on statistical phylogenetics, serves to eliminate such techniques from the procedural repertoire of practising taxonomists, whose mathematical literacy, more often than not, stops short of theoretical statistics.

It has been my aim to try to bridge the gap between the intuitive appeal of techniques such as parsimony, and the valuable, but complex, methods of maximum-likelihood estimation, by developing a suite of robust QND (Quick 'N Dirty) methods. QND methods have a number of advantages: they are easily applied (this is especially important for large data sets); they provide good heuristic approximations to more stringent techniques, and they do so under a wide range of initial conditions. All of the weighting or optimality criteria I propose can be carried out with only a hand calculator. This, to me, is of real value.

CHAPTER 3

TWO OPTIMALITY CRITERIA FOR SELECTING SUBSETS OF MOST-PARSIMONIOUS TREES

A manuscript submitted to Systematic Zoology

INTRODUCTION

In cladistic analysis, the phylogenetic relationships of Operational Taxonomic Units (OTUs) may be estimated by using the criterion of maximum parsimony, in which a tree with the minimum number of character transformations is taken to represent the best hypothesis of these relationships (Kluge and Farris, 1969). If all characters in a given character-OTU array are good indicators of phylogeny, the resulting minimum-length tree will have a length that is close to the total number of derived character states (i.e., the tree consistency index, defined by Kluge and Farris, will be close to 1). However, in most cases, there will be a subset of characters that is not strictly contingent on ancestry, but represents either adaptations, or reflections of structural plasticity. Such characters may be incompatible (*sensu* Estabrook 1984:149) with ancestrally-determined characters, and an analysis in which they are used often results in a suite of most-parsimonious trees each exhibiting some degree of homoplasy.

A researcher, faced with multiple most-parsimonious trees, can do one of three things: present all such trees as hypotheses of phylogeny; review all trees and select the best tree (on the basis of informed speculations and a priori expectations concerning the relationships and stabilities of the characters); or use an alternative optimality criterion in addition to that of minimum length. The first of these options can be used if the number of most-parsimonious trees is small (< 5). However, with large sets of minimum-length trees, it will be impractical and uninformative.

Alternatively, a researcher may decide to pursue the second course of action and select a tree on the basis of speculations about "most probable" relationships. In many cases, however, there is little or no prior knowledge about plausible OTU relationships, or even about the stability of the characters used to define such relationships.

In such circumstances, the most appropriate course of action is to use an alternative optimality measure (or index) that makes no *a priori* judgement about the value of the characters themselves, but rather selects a tree that possesses, in addition to maximum parsimony, some other desirable property. Two such measures already exist: Farris's (1972) *F*-value, which is essentially a measure of phenetic similarity; and the *D* measure of Brooks et al. (1986), which selects a tree that optimises information regarding phylogenetic constraints.

In this paper, I describe two new optimality indices. The first, the Optimal Character Compatibility Index (OCCI), is based on compatibility

methods (Estabrook *et al* 1977; Meacham, 1980), and selects the tree (or trees), from a set of most parsimonious trees, that has the largest number of compatible characters. The second index, the Optimum-Likelihood Index (OLI), selects the tree(s) which is most likely (relative to other most parsimonious trees) to give the observed character-OTU data, as a representation of *evolutionary history*. I also report the results of simulation trials in which both indices almost always retrieve the tree that most closely resembles the "true" tree. Finally, I discuss the rationale and advantages in using the OCCI and OLI, over other supplementary optimality criteria.

Terminology and Definitions

Cladograms or phylogenetic trees represent estimates of phylogenetic relationships between the Operational Taxonomic Units (OTUs) of a study. Most-parsimonious trees and minimum-length trees are taken to be equivalent in this paper. Trees selected using the OCCI and OLI will be called Optimal Character-Compatible (OCC) trees and Optimum-Likelihood (OL) trees, respectively.

In this paper, only discrete binary or multistate characters are considered. A character is said to be (fully) consistent if and only if, the number of times its character states appear as apomorphies is equal to the minimum number of transformations it can have [the minimum number of transformations a character can have is called its range (Farris, 1969), and is equal to the number of character states minus one].

For any given tree, and for any given character, the number of hypothesised character state changes is called the length of that character, and the ratio of character range to length is a measure of the unit character consistency. The higher this measure, the fewer times the character has changed relative to the number of states.

Two characters are said to be compatible if a tree exists on which both are fully consistent. This notion of pairwise compatibility can be extended to include groupwise compatibility.

THE OPTIMAL CHARACTER COMPATIBILITY INDEX (OCCI)

For any tree, in a set of most parsimonious trees, the OCCI is calculated simply as the ratio of the number of characters with a consistency of one to the total number of characters:

$$OCCI = n_1/N$$

where n_1 is the number of characters that change only once; and

N is the total number of characters.

The OCCI exists in the interval [0,1]. OCCI is maximised when all characters change only once, i.e., there are no ambiguous data.

In effect, the OCCI is a measure of the size of the largest clique, in relation to the total number of characters. Expressing the OCCI as a ratio allows comparison of the index among data sets; obviously, for a suite of trees, generated from the same data set, the OCCI of each tree can be measured simply by counting the number of compatible characters hypothesised for that topology.

The tree that maximises the OCCI is selected as the OCC tree. In some instances, a subset of trees is retrieved. It may be desirable, in such situations, to compute a sequential OCCI, in which a subset of trees is selected, for which the number of characters that have changed only once are maximal; from this subset, trees that maximise the number of characters that have changed twice are chosen, and so on. The simplest and most informative way of expressing a sequential OCCI is:

$OCCI_1 = n_1/N$, $OCCI_2 = n_2/N$, ..., etc. The subscripts on the OCCI indicate the number of changes for which the clique of characters is being considered. When only the largest clique of compatible characters is considered, the OCCI is not indexed by a subscript.

THE OPTIMUM-LIKELIHOOD INDEX (OLI)

The OLI estimates the likelihood that a particular tree, and its particular arrangement of character transitions, will give the observed data (i.e., character-OTU distribution). It is derived from the maximum-likelihood (ML) method of estimation of "evolutionary history" (this method differs from Felsenstein's (1973) method in which only the topology of the tree is estimated). Its derivation is given in the Appendix to this thesis. It is calculated as:

$$OLI = \sum_{i=1}^{N} c_i \ln \bar{r}_i$$

where $\bar{r}_i$ is the expected number of changes of the ith character; and

c_i is the number of changes of character i, for a particular tree.

Tree(s) that maximise(s) the OLI are selected as OL trees.

When $\bar{r}_i$ is unknown, it can be estimated by taking the geometric mean of the number of changes for the ith character.

OLI exists in the interval $(0, \sum_i r_i \ln r_i]$.

Maximum-likelihood methods of phylogenetic reconstruction are statistical estimation techniques that incorporate assumptions about the rate and rarity of character changes into their calculations. It is not essential, under the ML model, to posit equal rates of change for all characters. As a simplifying assumption, however, it is assumed that branch lengths (i.e., lineage "lifetimes", or anagenetic periods) are approximately equal. When these assumptions are taken into account, it is quite easy to calculate, for

any given evolutionary history, the probability or likelihood of obtaining the observed character-OTU data. The tree for which this probability is maximised is taken as the ML estimate of phylogeny.

Felsenstein (1978) has argued, and demonstrated, that ML estimation of evolutionary history, as opposed to topology, does not meet the sufficient conditions for consistency, the property of an estimate which guarantees that as more data are added the estimate converges to the true value. This is because estimation of evolutionary history requires that an estimate be made of the pattern of character changes for each character. As "infinitely many" characters are added (i.e., as the data set becomes very large), there are infinitely many parameters that need to be estimated, thus violating one of the sufficient conditions for consistency. This does not, of course, mean that the estimate will not be consistent, only that we cannot say for sure that it will be.

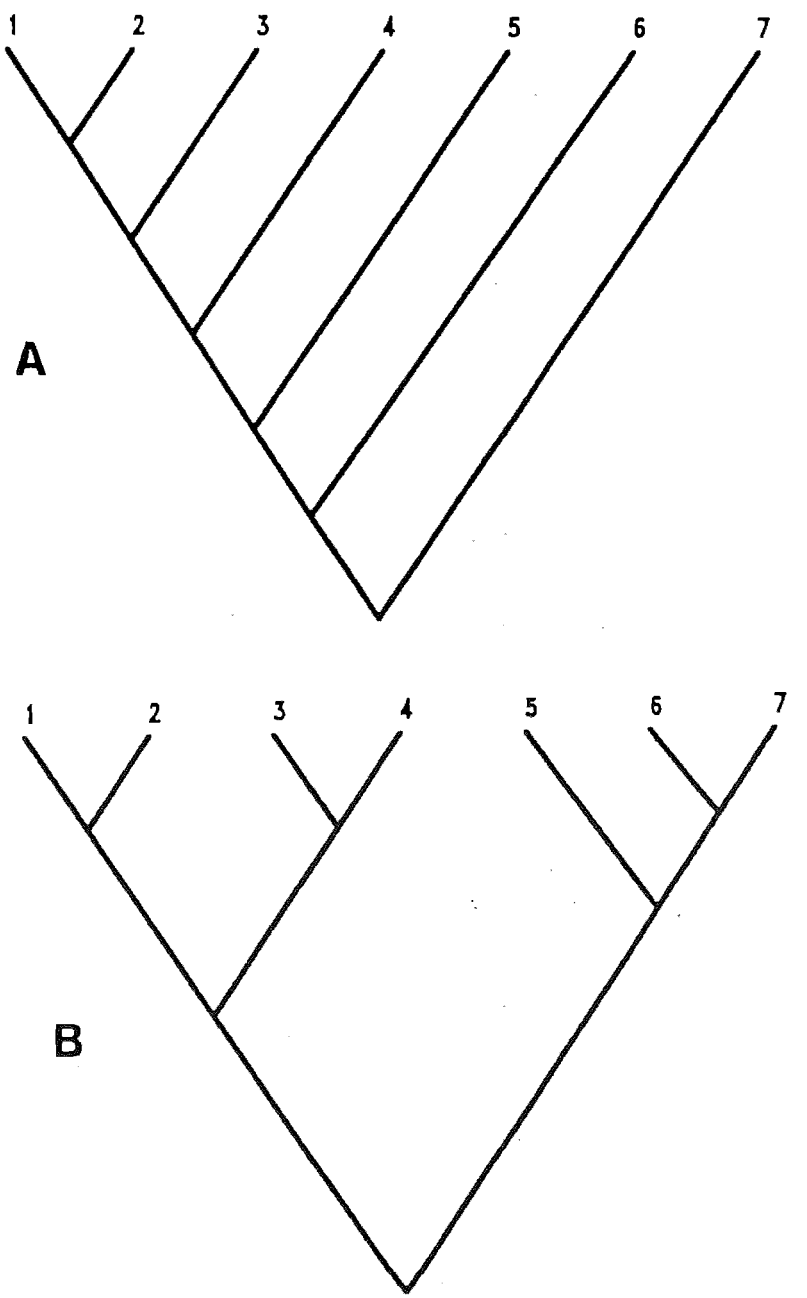
However, even though there is no guarantee that the OL estimate will be consistent, the OL tree does have intuitive appeal because of the fact that differential rates of character change are taken into consideration. Also, as I will show in the next section, the OLI performs well in simulation trials.

SELECTION OF OCC AND OL TREES USING SIMULATED DATA

Clearly, optimality criteria that are used in addition to maximum parsimony should have at least two properties: their use should result in the selection of a smaller subset of trees; and they should optimise the representation of some biologically significant factor (for example, phylogenetic constraints, or phenetic similarity). Furthermore, the use of an optimality criterion can contribute significantly to a cladistic analysis if the criterion in question allows the selection of trees (from a set of most-parsimonious trees) that most accurately resemble the true tree, or are the closest approximations to it. Until now, the degree to which optimality criteria can identify the true tree, from a set of minimum-length trees, has not been tested.

In order to test the ability of both indices to identify the "true" phylogenetic tree from a set of most-parsimonious trees, 30 simulations were performed. Data sets with 7, 11 and 20 OTUs were used (the number of OTUs does not include the "hypothetical ancestor" which was used to root the tree). The true tree for each of these data sets was one of two topological types (Fig. 3.1). The true topology of each fully bifurcating tree was defined by a set of compatible binary (0,1) characters. Every monophyletic group of OTUs was defined by 1 or 2 synapomorphies. Table

Figure 3.1 Examples of the topologies of the "true" phylogenetic trees used to generate the simulation data sets. (A) Maximally asymmetrical tree (i.e., Hennigian comb topology). (B) Maximally symmetrical tree.



2 gives details of the data sets used.

Random characters (also binary) were generated using a BASIC program on an IBM-PC. The probability that each character state would be included in the character vector was 0.5. For each data set, the proportion of random characters varied from one-sixth to twice the number of consistent characters. These characters were appended to the OTU-character array. All characters of "hypothetical ancestors" were coded 0.

PAUP (Swofford, 1985) was used to analyse the data. The options used to find the most-parsimonious trees were HOLD=10 SWAP=LOCAL. In addition, the CHGLIST option was used to obtain unit character consistencies, and the FVALUE and STATS options were used to obtain the value of the *F* statistic and Consistency Index of each tree.

For some data sets, the true tree was not one of the most-parsimonious trees. The congruence of these trees, and of the subsets identified by the OCCI and OLI, with the true tree was assessed by counting the number of matching monophyletic groups or components (Nelson and Platnick, 1981; Simberloff, 1987) between the two. (The tree in Fig. 3.2 has five non-trivial components: (a,b), (d,e), (f,g), (a,b,c), and (d,e,f,g). For any fully bifurcating tree, there are t-2 components, where t is the number of OTUs. Two components are said to match if their sets of OTUs are the same).

Table 3.1 summarizes the results of the test. Of the 30 data sets used, 21 produced more than one minimum-length tree. Of these, one data set (Data set 27) resulted in an unresolved trichotomy, which was represented by three trees. In *all* other analyses in which more than one minimum-length tree was found, OCC trees were identical to the true tree topologically, or had the highest number of components in common with the true tree. OL trees shared the highest number of components with the true trees in all but one of the 20 applicable analyses. In contrast, trees with the lowest *F* statistic identified the true tree or the tree with the largest number of identical components in only 11 of the 20 trials in which multiple minimum-length trees were found.

DISCUSSION

As stated earlier, optimality criteria should meet at least two requirements: they should identify a (small) subset of trees from the complete set of most-parsimonious trees; and the selected tree(s) should optimise some factor that has biological relevance. The first of these requirements is obvious. The second may need some explanation.

A phylogenetic tree is effectively a model (or high-level hypothesis) of relationships between OTUs. The model is built up from lower-level

Figure 3.2 The components of a hypothetical phylogenetic tree. Component 1=(a,b); Component 2=(d,e); Component 3=(f,g); Component 4=(c,(a,b)); Component 5=((d,e),(f,g)). The final component ((c,(a,b),((d,e),(f,g))) is trivial as it includes all members of the group.

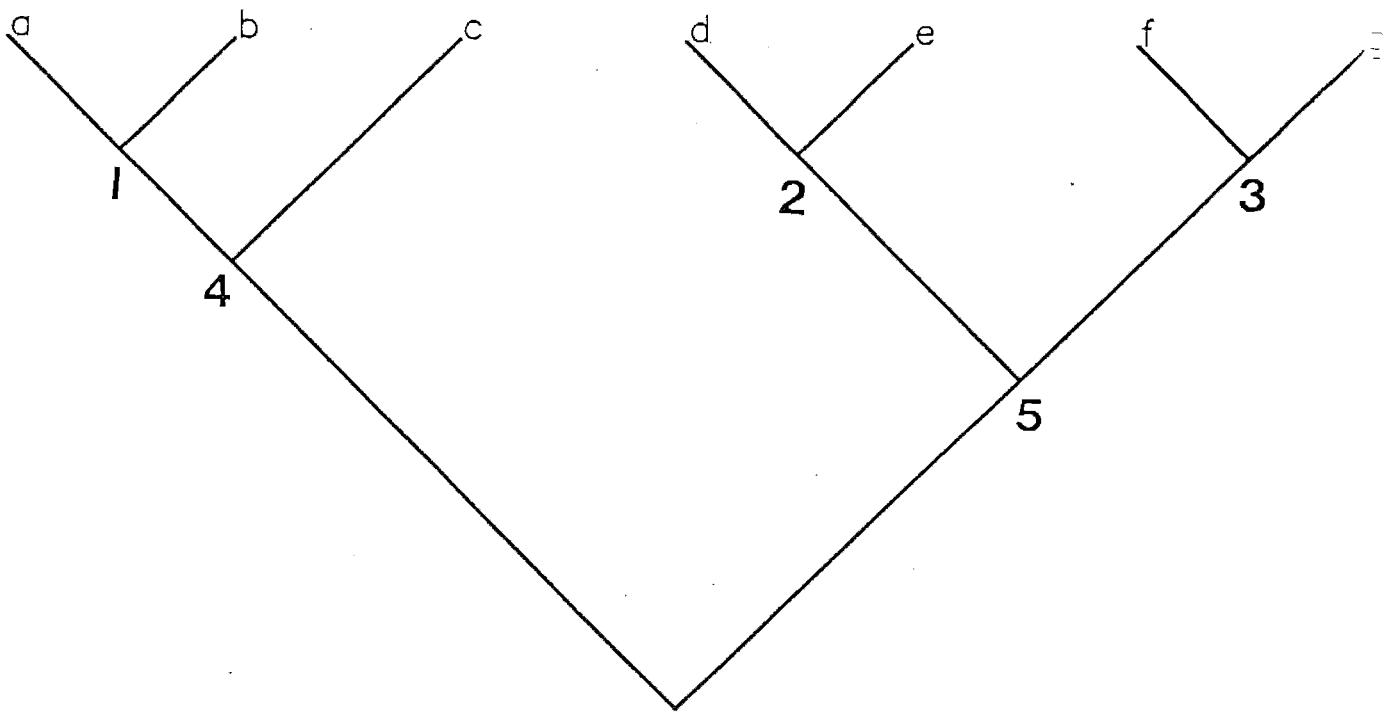


Table 3.1 Results of the simulation trials. The number of OTUs includes the hypothetical ancestor. The asterisk (*) indicates that the tree selected by an index most closely approximates the true tree.

Data set	Tree type[a]	Number of OTUs[b]	Number of consistent characters	Number of random characters	Number of minimal-length trees	Consistency Index	Tree Length	OCCI[c]	OLI[c]	F[c]
1	A	7	12	6	1	0.667	27	-	-	-
2	A	7	12	6	2	0.621	29	0.667(2)*	18.24(2)*	0.188(4)
3	A	7	12	6	2	0.600	30	0.667(2)*	20.00(2)*	0.180(4)*
4	A	7	12	10	2	0.564	39	0.545(5)*	27.23(5)*	0.261(4)*
5	A	7	12	10	1	0.550	40	-	-	-
6	A	7	12	23	5	0.486	72	0.343(4)*	56.88(4)*	0.300(4)*
7	B	11	10	3	8	0.591	22	0.769(9)*	15.86(9)*	0.255(7)
8	B	11	10	6	2	0.516	31	0.563(8)*	26.25(8)*	0.311(5)
9	B	11	10	9	1	0.463	41	-	-	-
10	B	11	10	12	4	0.440	50	0.318(5)*	47.29(5)*	0.399(5)*
11	B	11	10	15	1	0.368	68	-	-	-
12	B	11	10	18	4	0.384	73	0.250(6)*	76.93(6)*	0.509(4)*
13	A	11	10	3	3	0.565	23	0.692(7)*	17.56(7)*	0.330(7)*
14	A	11	10	6	4	0.516	31	0.375(4)*	23.52(4)*	0.330(3)
15	A	11	10	9	1	0.487	39	-	-	-
16	A	11	10	12	1	0.468	47	-	-	-
17	A	11	10	15	2	0.379	66	0.200(3)*	71.01(2)	0.392(3)*
18	A	11	10	18	1	0.378	74	-	-	-
19	B	20	19	6	8	0.417	60	0.600(14)*	72.43(14)*	0.406(12)
20	B	20	19	12	5	0.320	97	0.419(12)*	138.87(12)*	0.686(10)
21	B	20	19	18	2	0.272	136	0.216(6)*	203.83(6)*	0.718(5)
22	B	20	19	24	1	0.253	170	-	-	-
23	B	20	19	30	4	0.237	207	0.224(10)*	336.87(10)*	1.037(10)*
24	B	20	19	36	1	0.221	249	-	-	-
25	A	20	19	6	6	0.410	61	0.520(11)*	72.60(11)*	0.376(11)*
26	A	20	19	12	8	0.32	97	0.355(9)*	136.30(9)*	0.801(9)*
27	A	20	19	18	3	0.276	134	Not applicable - unresolved trichotomy		
28	A	20	19	24	2	0.247	174	0.116(3)*	271.35(3)*	0.804(3)*
29	A	20	19	30	6	0.231	212	0.061(2)*	335.92(2)*	0.836(2)*
30	A	20	19	36	2	0.224	245	0.055(2)*	393.13(2)*	0.931(2)*

[a] Tree types A and B refer to the tree topologies illustrated in Figure 1.
[b] The number of OTUs does not include the hypothetical ancestor.
[c] Values in parentheses indicate the number of matching components between the selected tree and the true tree. Asterisks indicate that the selected tree is the best matched tree in the set of minimal-length trees.

hypotheses - hypotheses about characters and biological processes. The best model is one that incorporates these postulates and minimises the number of *ad hoc* hypotheses required to "explain away" logical conflicts, that may arise between the postulates (Farris, 1983). By definition, optimality criteria are used to choose phylogenetic models of "best fit". Maximum parsimony is itself an optimality criterion (Swofford and Berlocher, 1987).

Both the OCCI and the OLI can be used in addition to the maximum parsimony criterion for selecting phylogenetic trees. The trees selected, therefore, incorporate all the features -both philosophical and operational - of most-parsimonious trees. However, OCC trees and OL trees have other desirable features.

The OCCI, by identifying trees with the largest clique of fully consistent characters, represents a "hybrid" of compatibility and parsimony methods. This is explained in more detail below.

Characters are low-level hypotheses: those used by systematists, are chosen because it is assumed *a priori* that they are reasonably good indicators of the "natural groupings" or monophyly of OTUs. Every character separates OTUs into as many groups as there are character states. Each member of a clique of compatible characters partitions the OTUs into groups which reinforce or subdivide OTU-partitions formed by other members of the clique. Incompatible characters provide two or more mutually-exclusive partitions of the OTUs.

In any character-OTU data set, there may be a proportion of characters that are not good indicators of ancestry. These characters, or more precisely their character states, may be dependent on ecological (including functional and epigenetic), ontogenetic, or accidental factors, none of which are necessarily influenced by phylogeny. Phylogenetic reconstruction using the maximum parsimony criterion treats all characters *a priori* as equal indicators of ancestry. However, one would expect the compatibility of these characters with phylogenetically-dependent characters and with each other to be low (Meacham and Estabrook, 1985).

Most parsimonious trees are those in which the number of conflicts of evidence of relationship (measured in terms of homoplasious character transformations) is minimised across all characters. They *do not* minimise the number of conflicting characters, however. Therefore, we still have to make a number of *ad hoc* statements about the value of particular characters as indicators of ancestry. Consider Trees 1 and 2 in Table 3.2. Assume that both are most-parsimonious trees. In Tree 1 two characters are fully consistent, whereas six are not. In Tree 2, there are three fully

Table 3.2 Hypothetical sets of the number of character changes for two trees. Both trees have the same length, but Tree 2 has the largest clique of compatible characters.

	Number of character changes									
Trees	1	2	3	4	5	6	7	8	OCCI	OLI
1	1	1	2	2	3	4	4	5	0.25	1.66
2	1	1	3	3	4	1	4	5	0.38	1.50

consistent characters, and five inconsistent characters. If the *ad hoc* statements mentioned above are of the form "Character x is a poor indicator of ancestry", we need to make six such statements for Tree 1, and only five for Tree 2. Tree 2 not only minimises the number of character transformations (maximum parsimony), but also has the largest clique of compatible characters. In other words, an OCC tree hypothesises an optimal number of ancestrally-determined characters, i.e. it optimises the fit of the model (in this case, Tree 2) to the *a priori* assumptions of the systematist.

The OCC tree has other properties. First, it is *taxonomically efficient*, by which I mean that it has the highest number of clades (or components) that can be identified uniquely by a single character. This property follows naturally from maximising the OCCI. Taxonomic efficiency should be distinguished from what Farris (1980) has termed *diagnostic efficiency*. The latter relates to the number of diagnostic statements required to retrieve the character-OTU matrix, completely. Taxonomic efficiency, on the other hand, refers to the ease with which we may identify taxon-membership, *in practice*. There is an obvious practical benefit in selecting the most-parsimonious tree in which we can identify the group-membership of an OTU on the basis of single characters.

Finally, the OCCI is easily applied. A list of unit character consistencies for each character can be obtained easily with many computer packages. Selection of the OCC tree can be done quickly by visually inspecting these consistencies. Ease-of-use is not, as some might suggest, a trivial property. Multiple most-parsimonious trees occur commonly and a quick and easy selection procedure is desirable.

OL trees are supported on the basis of their statistical properties, i.e., their relationship to maximum-likelihood (ML) estimates of phylogeny. As an alternative to other tree reconstruction methods, and depending on how well the assumptions of the ML model represent reality, ML estimation has some desirable features such as efficiency and consistency of estimates (although these may not apply to the ML model given here; see discussion above). ML estimation is also intuitively appealing because it takes account of the rate or probability of character change.

In the *true* phylogenetic tree, characters with a higher rate of change will, on average, have more character transformations. If we have no prior knowledge about what these rates of change might be, the likelihood of a particular evolutionary history must be calculated over the range of possible rates of change. If we can use the method of maximum parsimony to estimate the true tree, then it follows that we can also hypothesise about

the expected number of changes for each of the characters used. The set of most-parsimonious trees, and the contingent character changes, give a clue as to what the expected number of changes might be, for each character. By taking the geometric mean of character changes hypothesised by each tree, we are effectively approximating the likelihood over the range of possible transformation rates. Note that the OLI is maximised when the hypothesised numbers of character changes, over all characters, is closest to our expectations about what these numbers should be. In using the OLI, we are therefore stating our preference for the tree that best represents our expectations.

A final comment on the relationship of the OCCI and OLI: as Felsenstein (1981,1984) notes, when some characters have a much higher rate of change than others, compatibility estimates of phylogeny equate with those of maximum-likelihood. This is because the compatibility of plastic or random characters with other characters is likely to be low. In contrast, ancestrally-determined characters are often fully consistent with each other. In reality, just as with simulated data, the chance of obtaining a clique of fully compatible random characters *larger* than that of ancestrally-determined characters is small. Unless the proportion of some characters, which are, say, ecologically dependent (i.e. adaptive), is larger than that of the indicators of ancestry, the largest clique will consist of the latter type.

It is not really surprising, therefore, that in simulation trials, both the OLI and OCCI were highly successful at retrieving the true tree (or the closest approximation to it), from a set of minimum-length trees. Felsenstein (1984:187) suggested that for compatibility measures to work well, the proportion of characters with high rates of change should be low. However, according to the results of my simulation trials, it seems that this is not necessarily true. The OCCI retrieved the best approximation to the true tree, even for those data sets with proportions of random characters (i.e., those with a relatively rate of change) as high as 0.67. In fact the results indicate that both OL and OCC trees are robust estimates of the true tree.

SUMMARY

Two new indices are proposed: the Optimum Character-Compatible Index (OCCI), based on compatibility methods, and the Optimum-Likelihood Index (OLI), based on maximum-likelihood estimation.

The OCC tree is one of a set of most-parsimonious trees in which the greatest number of characters change only once.

By virtue of the fact that they have the largest clique of compatible

characters, OCC trees have the following properties:

1. They require the smallest number of *ad hoc* statements concerning the reliability of characters as indicators of ancestry.

2. They are taxonomically efficient, in that they have the highest number of components that can be identified by a single character.

Amongst most parsimonious trees and their contigent hypotheses of evolutionary history, OL trees have the highest probability of giving the observed data.

Application of both the OCCI and OLI in simulation trials almost always resulted in the retrieval of trees that were identical, or very close approximations, to the true phylogenetic trees.

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CHAPTER 4

AN INFORMATION-RICH CHARACTER WEIGHTING PROCEDURE FOR PARSIMONY ANALYSIS

A paper published in New Zealand Natural Sciences, 16:97-103.

"With a true view, all the data harmonize, but
with a false one the facts all clash."

Aristotle
Ethics

AN INFORMATION-RICH CHARACTER WEIGHTING PROCEDURE FOR PARSIMONY ANALYSIS

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ABSTRACT

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A weighting procedure is proposed which takes account of prior information pertaining to the characters used in a parsimony analysis. This information comes from specific knowledge about the biology of the group in question, as well as general evolutionary theory. The weighting procedure consists of three stages: (1) an initial parsimony analysis followed by (2) an examination of the character consistency indices and associated character weights, with reassignment of weights based on prior knowledge of the group; and (3) a reanalysis using the weighted data. The procedure is an iterative one, and can be terminated once the resultant tree has converged to a "constant value", or after a predetermined number of runs. The resultant tree may or may not be as short as the most parsimonious tree. It is argued that in taking account of prior information, the proposed procedure is information-rich (IR). Finally, the procedure is shown to be one of a family of IR techniques which are commonly used in parsimony analysis.

KEYWORDS: information-rich - character weighting - parsimony - phylogeny.

INTRODUCTION

The application of character weighting procedures in taxonomic analysis has always been a contentious issue, particularly for phylogenetic systematists. Systematists try to remove personal bias from their taxonomies by developing "objective" methods of classification. However, every systematist accepts that there are always some characters which are less "reliable" as indicators of phylogenetic relationships than others. Convergent characters may evolve in distantly related groups, either as a result of similar environmental pressures, or random genetic drift. Characters may also be misclassified through some error of interpretation on the part of the taxonomist. It seems clear that for any analysis which attempts to determine the phylogenetic relationships between groups of organisms, these characters should be given a low weight relative to those which are good indicators of ancestor-descendant relationships. However, the realisation that this must be so does not make the task any easier. Two problems arise:

1) how can these characters be identified; and

2) how can character weights be assigned to these and other characters, to reflect their *relative* phylogenetic information content.

Most systematists agree that procedures for character weighting, while essential, should rest on objective foundations. As a result, *extrinsic* character weighting procedures (i.e., those which use information not obtainable from the matrix of character states and taxa in question) have been rejected in favour of *intrinsic* methods which are more "algorithmic" and less susceptible to personal bias (see the methods developed in Farris (1969) and Penny & Hendy (1985)). Extrinsic weighting procedures are a special class of *a priori* weighting methods (*sensu* Neff 1986). By definition, extrinsic information precludes the

use of consistency indices, and compatibilities, both of which are obtainable from the character-taxa matrix, and are therefore items of intrinsic information. In this paper I will use the terms "prior information" and "extrinsic information" interchangeably.

The reason given for rejecting extrinsic weighting is that there is seldom any information available to determine which characters are good indicators of phylogeny in the group being studied. This is only partially true: while we cannot assign absolute weights (i.e., interval or rational values) to all characters, there is always some qualitative information available on the relative value of some characters in the data set. This information can be elicited from research on the comparative biology of the taxa in question, as well as from a general theoretical framework of population and evolutionary biology. So-called "objective" methods do not incorporate such information, and proponents of these methods are prepared to sacrifice prior information for objectivity.

In this paper, a method is presented which takes account of prior information while at the same time preserving the objectivity of intrinsic techniques. For this reason, the method is called an information-rich (IR) weighting procedure.

As a method, IR weighting is primarily an algorithmic extension of the principles discussed by Neff (1986) (and anticipated by Hecht & Edwards (1976)) in relation to *a priori* character weighting. Furthermore, I will argue that it is, in fact, one of a family of procedures which are commonly used in phylogenetic analysis.

I have applied IR weighting with parsimony analysis, but the method is general enough to be applied to all phylogenetic procedures with only minor modification.

TERMINOLOGY

Phylogenetic analysis attempts to uncover the evolutionary relationships between groups of study organisms or *evolutionary units* (EUs). These relationships are often displayed as a branching diagram known as a *phylogenetic tree* or *cladogram*.

For each EU, systematists have at their disposal information pertaining to the characters which may be used to identify the EU. Care must be taken to distinguish between *characters* and *character states*: character states refer to the "values" of a particular character, e.g., the character "hair-colour" has "brown","black", "blond", and "red" as its character states. For computational purposes, then, each EU may be represented as a set of character states. The number of possible character state changes is known as the *range* of a character. The range of a character is equal to the number of character states minus 1. For any given tree, the number of character state changes per character is known as the *length* of the character. The ratio of range to length is known as the *character consistency index*.

The problem of phylogenetic analysis can be stated thus:

Given what is known about the evolutionary process, how can EUs and character state changes be assigned to the terminal nodes and branches of a cladogram, respectively, to project a scientifically acceptable hypothesis of evolutionary history?

A number of phylogenetic methods have been developed, the most popular of which is *parsimony analysis*. Parsimony attempts to find the tree which has the fewest character state changes. The most parsimonious, or minimal-length, tree is taken as a hypothesis of evolutionary history. (Cladists argue that parsimony is based on a philosophically sound principle: the best hypothesis requires the fewest assumptions. Farris (1983), for instance, equates "phylogenetic tree" with "hypothesis" and "character state changes" with "assumptions". Hence, it follows that minimising character state changes on a phylogenetic tree is equivalent to choosing the best scientific hypothesis. In the last section, I will argue that this is not necessarily true).

METHOD

IR weighting is a three-stage process:

1) A parsimony analysis is conducted, *without* weighting.

2) Characters are ranked on the basis of their consistency indices. The user examines the ranks of these characters, and changes those which conflict with prior information. As stated earlier, this information may take the form of biological principles, theoretical considerations, ontological and genetic evidence, as well as the shared expec-

tations of other systematists working on the same group of organisms.

3) A weighting criterion is applied, using the revised ranks (and the consistency indices corresponding to these ranks), and the analysis is repeated. This process continues until the resulting tree converges to some stable value, or after a predetermined number of iterations.

Each of these stages is discussed in more detail in the following section, and will be illustrated with reference to the hypothetical data set of a group of potentially interbreeding but geographically isolated sub-species of parasitic flukes and their character sets, given in Table 1.

AN ILLUSTRATIVE EXAMPLE

Stage 1

A parsimony analysis is conducted using the data. A number of computer packages are available for this analysis (e.g., PAUP (Swofford 1985) and PHYLIP (Felsenstein 1987)). The output of the analysis should include the number of hypothesised changes for each character. From this, we can calculate the consistency index of the ith character, c_i

$c_i = r_i / l_i$

where r_i is the range of character i, and

l_i is the number of hypothesised changes of i (i.e., its length).

For the hypothetical data, parsimony analysis results in the tree shown in Fig. 1a.

This stage is no different from any other intrinsic weighting procedure, in that it involves an initial exploratory analysis.

Stage 2

The character consistency indices are ranked in descending order, i.e., the highest consistency index is given a value of 1, the next highest, a value of 2, etc. In Table 2, these ranks are given in column 5.

Once these ranks are available, the systematist is able to examine the *hypothesised relative stability* of the characters, and reassign ranks in accordance with what prior information is available. For instance, in the example, we see that Character 8 (follicular or whole testes) is hypothesised to have changed more often than most other characters in the group. However, it can be argued that changes in testicular morphology can lead to dramatic changes in reproductive biology, which in turn lead to reproductive isolation. Since the group is known to be at least potentially interbreeding (bearing in mind that the group in question is a hypothetical one), it seems likely that reproductive characters will, for the most part, be highly conservative. The same can also be said for Character 6 (genital opening, left or right). Certainly, biological theory would suggest that these characters are probably more conservative than characters related to the assimilatory system (Characters 4 and 5).

On this basis, it would be justified to reassign the ranks of characters 6 and 8 to the highest

Character			Taxa S1	S2	S3	S4	S5	S6	S7
1.	Body shape	(1 = elongate; 0 = elliptical)	1	0	1	0	1	0	0
2.	Body size	(1 = <5 mm; 0 = >5 mm)	0	1	1	0	0	1	0
3.	Head collar	(1 = present; 0 = absent)	1	1	1	0	1	0	0
4.	Oral sucker	(1 = terminal; 0 = sub-terminal)	0	1	0	1	0	1	0
5.	Gut caeca	(1 = diverticulate; 0 = smooth)	1	0	0	0	1	1	0
6.	Genital opening	(1 = left; 0 = right)	0	0	1	1	0	1	0
7.	Testicular fields	(1 = anterior; 0 = posterior)	1	0	0	1	0	1	0
8.	Testes	(1 = follicular; 0 = whole)	0	1	0	0	1	1	0
9.	Testes	(1 = lobed; 0 = complete)	1	0	0	1	1	0	0
10.	Eggs	(1 = with filaments; 0 = without)	1	0	1	0	0	1	0

Table 1. Hypothetical character-taxa matrix consisting of 6 subspecies (S1-S6) and 1 hypothetical ancestor (S7), and 10 characters of a group of parasitic flukes. The hypothetical ancestor serves to determine the evolutionary direction of the characters.

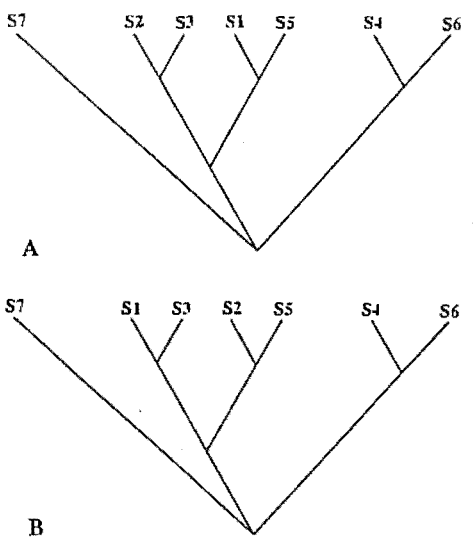

Figure 1. Phylogenetic trees derived using (A) unweighted and (B) weighted characters. The position of S1 and S2 differs in the two trees. (For consistency, the hypothetical ancestor, S7, has been positioned on a separate branch).

value, i.e., the rank of 1. Correspondingly, "new" consistency indices can be assigned to characters 6 and 8; in this case, the consistency index associated with rank 1 is 1.000. The new ranks are given in Column 6 of Table 2.

In essence, this stage involves the incorporation of information other than raw morphological data into the analysis. In practice, the taxonomist must be prepared to justify the reassignment of ranks, and the information which prompts such reassignment.

Stage 3

Consistency indices are reassigned in conjunction with rank reassignment because many weighting measures are functions of these indices. In this example, Farris' (1969) concave unbound-ed weighting function will be used. For the ith character, the weight, w_i is given by

$w_i = ((2n\text{-}3)c_i)^3\text{-}1.$

where n is the number of EUs.

These weights, applied to the reassigned consistency indices, are given in Column 7 of Table 2.

Stage 1 (a parsimony analysis) is repeated, this time using the weights given. The resulting tree is displayed in Fig. 1b, and the new consistency indices, and ranks, are given in Table 3.

Characters 6 and 8 now have ranks of 2. Clearly, this is more satisfactory than the previous scale, for the reasons mentioned above.

At this point, it is important to note that the unweighted length of this tree (i.e., the number of character changes, not corrected for weights) is one more than that of the tree derived in the initial parsimony analysis: the weighted tree is of length 22, while that of the unweighted tree is of length 21. Weighting has resulted in a tree which is not equivalent topologically to the most parsimonious tree, nor does it have the property of being a minimal-length tree (Fig. 1b). The consequences of this, and its justification, will be discussed in the next section.

The analysis is repeated, and the rank of Characters 6 and 8 are reset to 1, while all others

Character	Range	Length	Consistency index	Rank	New rank	Weight
1. Body shape	1	2	0.5	2	-	165
2. Body size	1	2	0.5	2	-	165
3. Head collar	1	1	1.0	1	-	1330
4. Oral sucker	1	2	0.5	2	-	165
5. Gut caeca	1	2	0.5	2	-	165
6. Genital opening	1	2	0.5	2	1	1330
7. Testicular fields	1	2	0.5	2	-	165
8. Testes	1	3	0.3	3	1	1330
9. Testes	1	2	0.5	2	-	165
10. Eggs	1	3	0.3	3	-	34

Table 2. Character consistency indicies, ranks, and weights derived from an initial parsimony analysis.

Character	Consistency index	Rank
1. Body shape	0.5	2
2. Body size	0.3	3
3. Head collar	1.0	1
4. Oral sucker	0.5	2
5. Caeca	0.3	3
6. Genital opening	0.5	2
7. Testicular fields	0.5	2
8. Testes	0.5	2
9. Testes	0.3	3
10. Eggs	0.5	2

Table 3. Character consistancy indicies and ranks after weighting.

adopt the new values of the weighted analysis.

Again Farris' weighting function is applied, and a parsimony analysis is conducted. The resulting tree, however, remains the same as that given in Fig. 1b. Similarly, the consistency indices of the different characters are the same as those given in Table 3. The analysis has "converged" to a single tree. This tree has desirable properties: the assignment of character state changes accords well with what is known about the biology of the group, and while it is not a minimal-length tree, it is only one unit longer.

DISCUSSION

To stress what was stated earlier, the systematist encounters two problems when attempting to weight characters for a phylogenetic analysis. The first of these concerns the differentiation of characters with a high phylogenetic information content from those with a low content. The problem is exacerbated by the fact that while we may have some knowledge about some characters, rarely do we have this kind of information about all characters.

The second problem is related to the first: how can a systematist assign weights to all characters when a) the appropriate weighting scale is unknown; and b) the phylogenetic content of only some characters is known (or can be guessed at).

The IR weighting procedure provides a solution to both these problems. First, it circumvents having to identify the phylogenetic information content of every character. Instead, by reranking the characters *after* an initial phylogenetic analysis, the systematist is free to decide on the *relative* reliability of only those characters for which there is any extrinsic information. The procedure therefore allows the initial analysis to determine the weights of those characters for which there is no information. Furthermore, decisions about relative stability (and consequently, relative weights) of characters are easier to make. It is easy to say, for example, that hair colour is less conservative than limb morphology, and at least as conservative as skin colour. It is more difficult, however, to assign an absolute weight to any of these features prior to an initial exploratory analysis.

Second, IR weighting frees the systematist from the task of selecting an appropriate weighting scale. Instead, all the systematist has to do is select one of a number of available weighting functions. Once this has been done, the reranking procedure will assign the appropriate weights to the characters. By reranking a character, IR weighting assigns a new consistency index to it. By doing this, a systematist is effectively stating the belief that the character can change as often as another with the same rank.

The scale of the weights is constrained by the choice of the weighting function. In the example given above, Farris' weighting function was used. However, there are a number of other functions available (Felsenstein 1981, Penny & Hendy 1985, Moody & O'Nolan 1987).

At this point, it should be noted that IR weighting can be used either as an exploratory procedure, or as a means of deriving a suitable tree. As an exploratory tool, IR weighting allows the user to compare the absolute length (as opposed to the weighted length) of the resultant tree with that of the tree prior to weighting. The absolute length of the weighted tree may be as short as, or even shorter than that of the unweighted tree. This is particularly useful when dealing with a large number of taxa (e.g., more than 20 EUs). This is because the procedures for obtaining the shortest possible tree become more cost-prohibitive as the number of taxa increases, and many computer packages resort to "best-approximation" methods.

Alternatively, a systematist may decide to

accept the weighted tree as the best hypothesis of evolutionary history, even though it is not the shortest tree. As in the example above, the weighted tree is considered to be a better hypothesis of evolutionary history because it incorporates more information about the characters than the unweighted tree does, at a "cost" of only 1 extra character state change. But can we justify not selecting the shortest tree as the best hypothesis of phylogeny ? What about Occam's Razor ?

At this point, it is worth reviewing the fundamental philosophy of parsimony analysis. When systematists use parsimony to construct hypotheses of evolutionary relationships it is rarely because they believe that evolution is parsimonious, i.e., that it proceeds with such a slow rate that all characters behave conservatively (Kluge 1984). Instead, parsimony is treated as a methodological tool, and as a way of constructing a hypothesis in a rational manner. Occam's Razor - "What can be explained by the assumption of fewer things is vainly explained by the assumption of more things" (Boehner 1957, translated by Kluge 1984) - is often cited as the fundamental motivation for the principle of parsimony in systematics. As stated earlier, cladists maintain that the minimal-length tree makes the least number of ad hoc assumptions regarding the multiplicity of character state changes.

This procedure is sound if there is no information about the nature of the characters selected. However, if information pertaining to the "conservativeness" of the characters is available from ontogeny, genetics or evolutionary theory, for example, then this procedure may falter. Consider, for instance, two characters, *a* and *b* for which there is a great deal of theory that indicates that the former is, in general, more conservative than the latter. However, after conducting a parsimony analysis, a systematist finds that, in the resulting tree, *a* has 3 changes while *b* has 1. While this may be the shortest tree for this data set, with the least number of ad hoc hypotheses, the character assignments it postulates is at odds with other theoretical considerations. To accept this tree would be to suggest that there exists an exception to the theory. If we accept that scientific theories are networks of hypotheses, theories, and observations, with each new theory or observation either supporting or casting doubts on others, it is important to realise that while we may have minimised the number of ad hoc assumptions for the tree itself, we have added one to the general body of biological theory. While it is true that exceptions abound in biology, many systematists would baulk at proposing such exceptions to the theory on the basis of what is really a hypothesis whose approximation to the truth is unknown (even, unknowable ?). A better tree would be one which preserved all relevant information, even at the cost of some units of length.

Finally, it should be noted that the weighting criterion proposed here is one of a family of information-rich procedures. Others in this set of procedures include Dollo parsimony (which has been formalised as a tree reconstruction procedure by Farris 1977), and the outgroup analysis of the polarity of character states (Watrous & Wheeler 1981).

Dollo's Law states that there is a smaller likelihood that complex structures would arise convergently, compared to simple structures. Dollo parsimony incorporates this by allowing only one forward change, while optimising the number of reversals. In Dollo parsimony, this information about the nature of character state change is supported by a background of evolutionary theory.

Outgroup analysis is a method by which ancestral character states may be determined by recourse to the distribution of these states in groups which are closely allied to the subject EU. It is argued that character states which are present in both outgroup and ingroup are likely to have been present in the ancestor of both groups. This information (which is not present in the EU-character matrix) allows the construction of a rooted tree, i.e., a tree which is not just a hypothesis of evolutionary relationships, but of evolutionary history.

I will conclude by noting that the techniques which are currently available for phylogenetic analysis are constantly being revised and enhanced so as to develop a family of procedures which take account of the diverse sources of information from which systematists must draw their conclusions. Information-rich procedures must be developed, but in such a way that these

methods are in harmony with intrinsic character weighting methods.

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REFERENCES

Boehner, P. (1957). *Ockham - philosophical writings*. Bobbs-Merrill. Indianapolis.

Farris, J.S. (1969). A successive approximations approach to character weighting. *Systematic Zoology* 16: 44-51.

Farris, J.S. (1977). Parsimony under Dollo's Law. *Systematic Zoology* 26: 77-88.

Felsenstein, J. (1981). A likelihood approach to character weighting and what it tells us about parsimony and compatibility. *Biological Journal of the Linnean Society* 16: 183-196.

Felsenstein, J. (1987). *PHYLIP (Phylogeny Inference Package) version 3.0 Manual.* University of Washington (distributed on floppy disks available from J. Felsenstein).

Hecht, M.K. & Edwards, J.L. (1976). The methodology of phylogenetic inference above the species level. In *Major Patterns in Vertebrate Evolution* (eds. M.K. Hecht, P.C. Goody, & B.M. Hecht). Plenum Press. New York.

Kluge, A.G. (1984). The relevance of parsimony to phylogenetic inference. In *Cladistics: Perspectives on the Reconstruction of Evolutionary History* (eds. T. Duncan & T.F. Stuessy). Columbia University Press. New York.

Moody, S.M. & O'Nolan, P. (1987). An *a priori* character weighting method for cladistic analysis. (Abstract) *American Zoologist* 26: 108A.

Neff, N.A. (1986). A rational basis for *a priori* character weighting. *Systematic Zoology* 35: 110-123.

Penny, D. & Hendy, M.D. (1985). Testing methods of evolutionary tree construction. *Cladistics* 1: 266-272.

Swofford, D.L. (1985). *PAUP: Phylogenetic Analysis Using Parsimony.* Illinois Natural History Survey. Illinois.

Watrous, L.E. and Wheeler, Q.D. (1981). The out-group comparison method of character analysis. *Systematic Zoology* 30: 1-11.

ADDENDA

A4.1 A prior character analyses

It is becoming increasingly apparent that *a priori* information is not the anathema of good systematic research. Consequently, a number of authors have proposed various ways of incorporating such information in a cladistic analysis (Hecht and Edwards, 1978; Gosliner and Ghiselin, 1984; Neff, 1986; Bryant, 1989, to name a few). One of the main themes to emerge from such studies is the paramount importance of character analysis (also see Chapter 1). There are at least five stages of *a priori* character analysis in any systematic study:

1. Selection of appropriate taxonomic characters. At the very least, this involves disregarding phenotypic features which are the result of accidents, those which are highly variable in all OTUs under consideration, and non-heritable characters (Blackwelder, 1967);

2. Identification of characters and character states. For instance, do the features "red petals" and "yellow petals" qualify as different characters, or as different states of the same character (Pimentel and Riggins, 1987)?

3. Identification of character states in OTUs. More often than not, two OTUs may share very similar but not identical features. In such situations, a decision must be made as to whether it is acceptable to treat both features as the same character state (Neff, 1986);

4. Determination of polarity and evolutionary sequence. At this stage, the plesiomorphic (= ancestral) state of a character is identified. If the character is a multi-state character, then the order in which the character states emerged in time should also be determined. In phylogenetic analysis, determination of the ancestral state is solved by Out-Group Analysis (Watrous and Wheeler, 1981), whereas character evolution may be inferred *a posteriori* by Transformation Series Analysis (Mikevich, 1981);

5. Decisions about the relative merit of each character as an indicator of monophyly. When numerical procedures are applied, this is equivalent to character weighting.

The first four of these stages have been covered extensively in the literature, and I will not deal with them here. Instead, I wish to comment briefly on how decisions about the relative "value" of taxonomic characters can be made.

I assert that a character is a good indicator of monophyly if it has a lower potential for change, and is consequently more stable. This is because it is more likely that taxa retain the ancestral states of such characters. This assertion, however, must be qualified with the following observation: if

we compare a two state character with a five state character, then it is obvious that the latter has a higher potential for change. However, it does not follow that it is a poorer indicator of monophyly, because each of its character states may identify a monophyletic group. Therefore, the *relative* value of a taxonomic character must take into account not only the absolute potential for evolutionary change, but balance this with the number of observed states. The inverse of Farris's (1969) consistency index is an example of a measure which takes the number of character states into account when quantifying the stability of a character.

The pattern of character change, itself, varies. First, there may be a sequential change, involving the progression from a plesiomorphic state to more advanced states, with each change being expressed as a new character state. Second, changes may involve only single "additions" of character states, but multiple secondary "deletions" (i.e., reversals). Finally, character change can include multiple independent "additions" (i.e., parallelisms or convergences) of identical character states, as well as "deletions" (Katz, 1987).

Traditionally, characters have been taken as hypotheses of homologies, i.e., if two OTUs have the same (derived) character state, then it is hypothesised that it is an inheritance of an ancestral state. However, if we claim that characters have a (potential) *rate of change*, and that some of these changes may be parallelisms, then we forfeit the notion that characters are hypotheses of homologies (unless we redefine each parallelism as a new character state). One cannot hold *a priori* that shared derived character states indicate common ancestry, while at the same time maintaining that they may also indicate parallelisms.

There are, of course, ways of inferring homology, e.g. by structural and ontogenetic equivalence (Patterson, 1982). When this can be done, it is unwise to admit hypotheses of parallelism [Dollo parsimony (Farris, 1977) provides a solution to the problem by allowing one "addition" and multiple "deletions"]. Often, however, ontogenetic evidence is not avaiable, so all that is left is structural equivalence (in parasite systematics, for instance, this is usually the rule rather than the exception). Even when this equivalence is exact, it is still possible that similar structures (or behaviours, or genetic sequences) may have arisen convergently. Under such circumstances, assigning a potential rate of change to a character is equivalent to replacing a hypothesis of homology with one of *morphogenetic tendency*, i.e., the tendency to develop similar structural features convergently.

The evolutionary rate of change of any character is a function of the ecological history of the clade, the character's structural integrity, and stochastic events, i.e,

$D(C) = f(E, S, R)$

where $D(C)$ is the evolutionary rate of change of character C;

E is a measure of ecological influence;

S is a measure of structural integrity; and

R represents random factors.

The ecological history of a group of organisms may influence the rates of change of some taxonomic features by means of natural selection. The rate of change of a character is also influenced by what I call its structural integrity (see Chapter 2, A2.3), a term that relates not only to a character's complexity but also to its relationship with other features of the organism. Finally, random genetic drift also influences the rate of change of characters, particularly those which are non-adaptive.

Traditional wisdom suggests that characters that are shaped more by ecological factors have a relatively higher potential for change (Bradley, 1986). However, there is no reason why this should be so. After all, if the ecology of an organism is "inherited" (i.e., if ancestors and descendents have invariant ecologies), then ecologically-determined characters can reflect evolutionary relationships very well. Similarly, complex structures are thought to be more conservative, because any change in them will involve drastic rearrangement of form and function, and as a consequence, result in severe maladaptation of the organism. However, this assumes that there is a correlation between phenotypic and genetic complexity, and that such a structure has high functional significance. These assumptions may not hold, particularly when such "complex" structures are controlled by one or a few genes (e.g., wild and vestigial wing types of *Drosophila* whose recombination ratios can be calculated using simple Mendelian single-locus laws). In such situations, there is no reason to believe that "complex" structures are less likely to change than "simpler" structures with equivalent adaptive value.

Attempting to decouple the influences of ecological history, structural integrity, and random events *a priori*, is difficult; even if it is possible, we can only guess what their *joint* influence is on the rates of character change.

It is possible, nonetheless, to come to some conclusion about the relative rates of change from character-OTU data. In order to do so, a link has to be made between character variability and potential rate of change.

It is reasonable to assume that if a character exhibits a high intra-taxon variability relative to other characters, then during the course of evolution, that character has the potential to be fixed in a number of states, whereas other characters with little or no intra-taxon variability will, most likely, perpetuate the dominant state (Farris, 1969). In other words, characters which tend to be polymorphic in taxa are likely to have high evolutionary rates of change. If it is also assumed that the processes that have lead to the observed character variability have not changed over time, we can infer that current estimates of character rates of change are indicative of past rates of change. [This is, of course, a very BIG assumption, and the only defence for making it is that it is standard practice in historical hypotheses (See Gould, 1985, on the principle of uniformitarianism)].

Another family of methods for arriving at *a priori* estimates of relative rates of character change involves the use of compatibilities (see Chapter 3 and 5 for a definition of compatibility). One of the simplest such measures is the proportion of taxonomic characters with which each character is compatible (Farris, 1969). Other measures have been proposed by Penny and Hendy (1985) and Moody and O'Nolan (1987).

Character variability analyses and estimation of character rates of change on the basis of compatibility are qualitatively different approaches. First, methods using character compatibility rely on the joint character-OTU matrix for deriving their weights, i.e., intrinsic information. The weights derived are unique to the set of OTUs in question, and contingent on the evolutionary history of that taxonomic group. Furthermore, there is, as yet, no way of representing the degree of intra-taxon variability in such weights.

On the other hand, investigations of character variability can use evidence from taxa other than the OTUs of interest (such information is called extrinsic information). This is often the case when one deals with "real world" taxonomic problems. The OTUs under consideration may be represented by single type specimens only, and these give no indication of character variability. However, evidence from other groups may offer clues about the relative stability of certain characters. (Note that other taxa may not have the complete suite of characters present in the in-group. For this reason, I emphasise the fact, in my discussion of IR weighting, that we usually know the value of a certain character relative to only a few others).

In effect, assessing the relative value of taxonomic characters by character-variability analysis can involve the use of indirect (but not unrelated) evidence. This by no means implies that such analyses are

subjective or arbitrary; on the contrary, by its very nature, character-variability analyses can only be carried out when we know something about the biology of the group in question.

Character-variability analyses and compatibility weighting are not mutually exclusive. Instead, when both methods arrive at the same relative character weights, they serve to reinforce our confidence in the resultant phylogenetic hypotheses. When there is disagreement between the corresponding character weights of the two methods, the taxonomist must decide which to use. IR weighting offers the taxonomist the opportunity to incorporate both sources of information into a phylogenetic analysis.

Finally, I will mention briefly an operational procedure I have developed for placing a qualitative "confidence level" on a phylogenetic hypothesis (the procedure is also described in Chapter 6). The procedure involves grouping characters to be used in a phylogenetic analysis in two categories: *well-defined characters* and *questionable characters*. Well-defined characters are those which meet the following criteria:

1. The character states are discrete, and can be identified without any doubt; and

2. Information on the occurrence of character states in all or most taxa is available.

Questionable characters are those which fail to satisfy at least one of these criteria.

If a phylogenetic analysis is conducted using some form of intrinsic weighting criterion [e.g., Farris's (1969) successive approximations method], then the reliability of a resultant weight for any character can be assessed on the basis of whether that character is well-defined or questionable. A phylogenetic analysis in which characters that are weighted highly are also those that were classified initially as questionable, would be treated with suspicion. This is because there is a relatively high likelihood that these characters and their occurrence in the taxa under consideration have been incorrectly reported or misinterpreted. On the other hand, if all highly weighted characters are also well-defined, then there is no reason to doubt the merit of the phylogenetic hypothesis. By following this procedure, a taxonomist can decide whether to place a high or low "confidence value" on a phylogenetic analysis, and act accordingly.

A4.2 A possible method for comparing an alternative phylogenetic tree against a set of most-parsimonious trees

It was mentioned earlier that a taxonomist might feel justified in accepting a less parsimonious phylogenetic tree if such a hypothesis

incorporates additional biological information. However, it is likely that, if such information comes from indirect sources (see discussion above), one would not want to stray too far from the most-parsimonious hypothesis of phylogeny, particularly if parsimony results in a good approximation of the true phylogenetic tree. The question, "How 'far' is 'far' ?" can best be answered statistically. Cavendar (1981) has outlined a method for testing whether the length of a given tree is significantly different from the most-parsimonious tree for four taxa. He also showed that as more taxa are added, the hypothesis test becomes increasingly inconsistent, i.e., it is more likely to reject the null hypothesis with a probability that differs from the pre-defined alpha-level (Felsenstein, 1984).

However, instead of testing whether the lengths of trees are significantly different from the most-parsimonious solution, I believe it is feasible to consider whether the distribution of character changes of an alternative phylogenetic hypothesis is different from a those of a set of most-parsimonious trees.

It is often the case that when there are inconsistencies in the character-OTU data, a number of most-parsimonious trees can be found. Each tree can be characterised by a set consisting of the number of changes for each character. Each set is equivalent to a collection of hypotheses about the stability of the characters used. Therefore, if a given tree, T_1, is to be compared to the set of most-parsimonious trees, it is reasonable to ask whether the hypotheses of character change under T_1 is consistent with those of the set of most-parsimonious trees.

For any set of most-parsimonious trees, two multivariate parameters may be estimated: the vector of mean number of changes for each character, **U**, and the variance-covariance matrix of numbers of character changes, **V**. Since we also know the vector of character changes for the tree to be tested ($\mathbf{C_T}$), we can use Mahalanobis' (1936) generalised distance, D^2, to quantify the difference between $\mathbf{C_T}$ and **U**:

$$D^2 = (\mathbf{C_T} - \mathbf{U})'\mathbf{V}^{-1}(\mathbf{C_T} - \mathbf{U})$$

Furthermore, it is possible to set an arbitrary cut-off point, against which D^2 may be compared; one such value is $\chi^2_{(n,0.95)}$, where n is the number of characters. (Note: D^2 is distributed χ^2_n, if the vector of numbers of character changes is multivariate normal. As this conditions will almost certainly not apply, the use of a χ^2_n distribution is simply a matter of convenience. Also, the invertibility of **V** depends on whether it is of full rank, i.e., the number of most parsimonious trees is greater than the number of characters. This is also unlikely. However, it is possible to use a

generalised inverse to estimate V^{-1}).

Under certain conditions, this procedure may tell us something about how closely an alternative phylogeny approximates the true phylogenetic tree, relative to most-parsimonious estimates. The emphasis is, therefore, shifted from *statistical inference* to *exploratory data analysis*. For instance, if the topologies of all most-parsimonious trees differ widely, then it is likely that a variety of character changes can be accomodated. This suggests that the most-parsimonious hypotheses are not necessarily any better in terms of its closeness to the true tree than one which is less parsimonious (within the limits imposed by D^2). If on the other hand, the variances of character changes is low, and the covariances of the number of changes between pairs of characters are relatively high, it suggests that only minor distortions in topology can be tolerated. It is also likely that increasing the number of changes in one character will lead to an increase in the number of changes of other characters as well. If character rates of change are assumed to be low, and covariances between characters are representative of population covariances, then the most-parsimonious trees will probably correspond closely to the maximum-likelihood tree (under the model proposed in Chapter 5). This is because the length of the former will be much shorter than the length of the next best estimate. Under such circumstances, a high value of D^2 may indicate that the alternative is not as good an estimator of the true tree as a most parsimonious tree.

The truth of these conjectures and the robustness of the criterion is unknown. I have identified these as future priorities of research.

CHAPTER 5

A FAMILY OF HEURISTIC METHODS FOR APPROXIMATING MAXIMUM-LIKELIHOOD SOLUTIONS TO PHYLOGENETIC TREES

A manuscript submitted to Journal of Evolutionary Biology

"All mathematical calculations about the course of nature must start from some assumed law of nature ... the doubt always remains - is the law true ? If the law states a precise result, almost certainly it is not precisely accurate, and thus even at the best the result, precisely as calculated is not likely to occur. But then we have no faculty capable of observation with ideal precision, so, after all, our inaccurate laws may be good enough."

Alfred North Whitehead

Introduction to Mathematics

INTRODUCTION

At present, a number of different numerical methods exist for reconstructing the evolutionary history of a group of organisms. Of these, the favoured statistical technique is based on maximum-likelihood (ML) estimation (Farris, 1973; Felsenstein, 1973, 1978, 1981, 1985; Barry and Hartigan, 1987). ML estimates are, in general, consistent and efficient i.e., they tend to approach the true value as more data are added, and for large data sets, they have a lower variance than other estimates. However, while ML estimation is a scientifically defensible protocol for selecting the evolutionary tree that best describes the history of a group of organisms, there is a significant practical problem with the use of such techniques. Thus, although there have been major theoretical advances in ML estimation techniques (Hendy and Penny, MS), there is a dearth of readily available computer programs with which to compute ML estimates of evolutionary trees (Felsenstein's PHYLIP package is the only one I know of) and, as a consequence, M-L estimation is not popular with most systematists. Furthermore, ML estimation is computationally complex, and the cost in terms of computing time is prohibitive. As a result, for large data sets, it is often more practical to resort to near-optimal or heuristic solutions.

In this paper, I review a family of methods which give good heuristic approximations to ML estimates of tree topologies when used in conjunction with common tree constructing techniques such as parsimony and compatibility, for which there are numerous programs available. Felsenstein (1978, 1981, 1984, 1988), in particular, has investigated the relationship between different tree reconstruction techniques and ML estimation. It is my aim, here, to review these and other heuristic methods, and outline the assumptions under which they can be used to generate M-L approximations. The methods I discuss are all relatively easy to use, and effectively bridge the gap between simple but sometimes unreliable techniques such as parsimony, and the more efficient but computationally complex ML procedures.

TERMINOLOGY AND ASSUMPTIONS

An *evolutionary tree* is a model of the history of branching or cladogenesis of the organisms under consideration. In this paper, only fully bifurcating trees are considered, i.e., trees with dichotomous branching only. I also assume that the aim of evolutionary reconstruction is to estimate the pattern of branching, or *tree topology*, only. However, under the constraints of the model developed here, it will be necessary to estimate the number of changes per character, and consequently, the pattern of evolutionary change. In effect, this is equivalent to developing a hypothesis of *evolutionary history*.

The uppermost or terminal nodes of an evolutionary tree comprise our subject organisms or *Evolutionary Units (EUs,* synonymous with *Operational Taxonomic Units, OTUs).* The internode

Table 5.1 Example of the character vectors of 3 hypothetical EUs.

A = {1,0,1,1,0}

B = {1,0,0,1,1}

C = {0,1,0,0,0}

distances (i.e., branch lengths) are taken to be proportional to the time intervals between speciation events (this assumption plays a part in the development of the model); a tree is said to be *rooted* if it is a representation of historical events, and not simply a hypothesis of relationships. A fully bifurcating, and rooted tree, has $2(m\text{-}1)$ branches, where m is the number of EUs. In this paper, the ith branch length is denoted by t_i. The internal nodes, of which there are m-2 in a fully bifurcating and rooted evolutionary tree, represent hypothetical EUs (*HEUs*). These are populations which are postulated to have existed just prior to a cladogenetic (or lineage-splitting) event. The lowermost (or basal) node is identified as HEU_0. Figure 5.1 illustrates this model of an evolutionary tree.

A systematist attempting to reconstruct the evolutionary history of a group of m organisms, has at his/her disposal information on the character states of each of n characters, for each EU. In this paper, I will assume that the characters are discrete and binary (i.e., there are only two character states coded 0 and 1). This assumption is made to simplify the development of the M-L model. The techniques discussed here, however, still apply to discrete multistate characters. Continuous characters may be recoded as discrete characters according to the protocols given by Archie (1985). EUs and HEUs can be defined/identified by vectors of n character states (Table 1). In order to develop a manageable likelihood function for the evolutionary tree, all characters will be treated as independent, each with its own constant rate of change at every branch. Furthermore, it will be assumed that changes are reversible, (i.e., characters can revert to a prior state), and that the probability of a change, whether it be 0->1 or 1->0 is constant and less than the probability of no character state change. For the ith character, the rate of change shall be denoted r_i, and the probability associated with a single change denoted as P_{iC}. The probability of no change in character states will be denoted P_{iNC}. Under this model, if two EUs share a common character state it is *not* assumed, *a priori*, that the states are homologous. This is because in hypothesising rates of character change, an implicit assumption is made that there is a finite probability that character states can arise more than once.

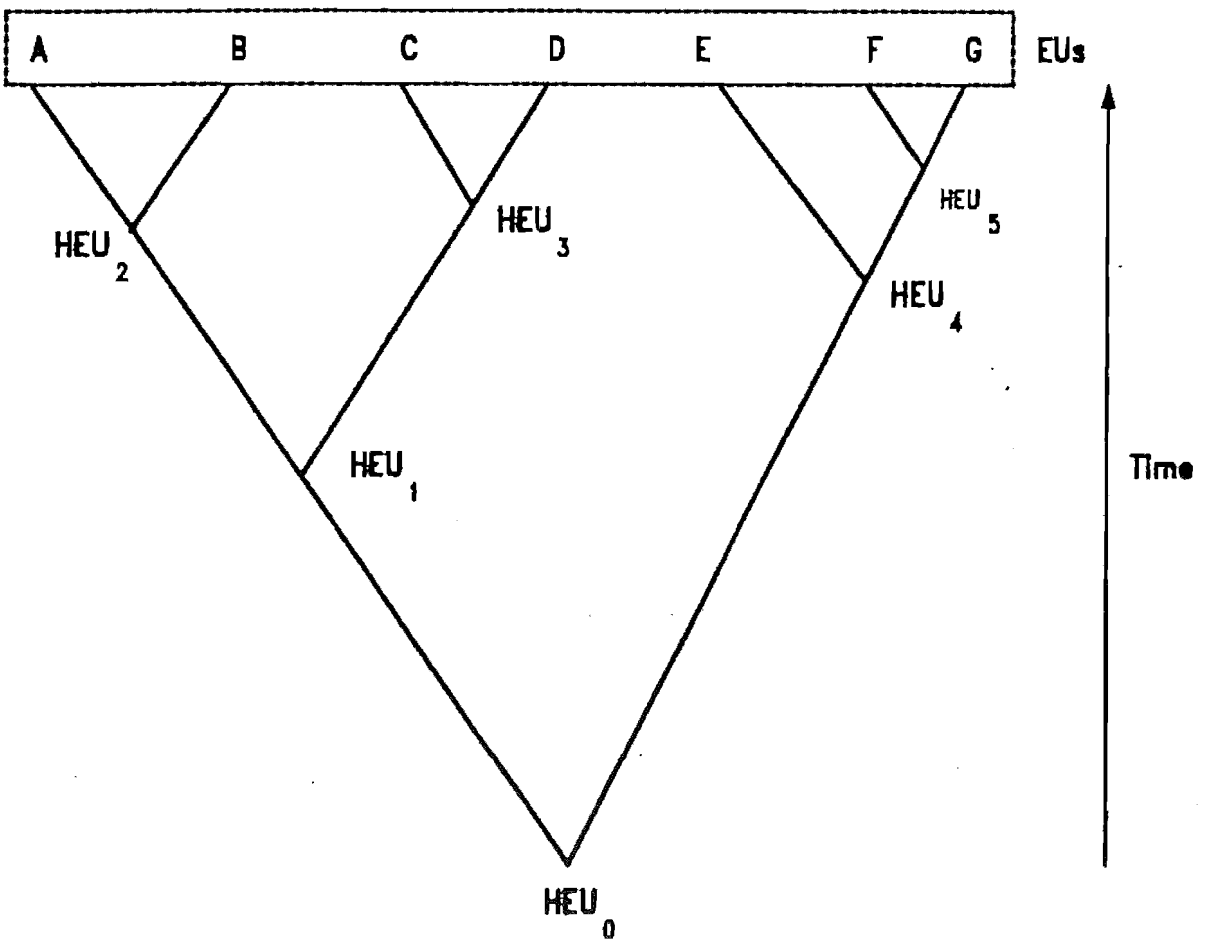
For any given tree, the ratio of the minimum number of character state changes (for n binary characters this is equal to n) to the hypothesised number of changes is known as the consistency index of the tree (Kluge and Farris, 1969).

The assumptions outlined here are reviewed in a later section.

DEVELOPMENT OF THE LIKELIHOOD FUNCTION

The likelihood function represents the probability that a particular tree topology will give the observed data, given the assumptions one makes about the evolutionary process, and the probabilities associated with character state changes. The tree whose topology maximises the likelihood function, has the highest probability of resulting in the observed character-EU distribution. In this model of cladogenesis, character change is assumed to be rare. Any change from one

Figure 5.1 A model of an evolutionary tree. The tree is "rooted", with *HEU0* as the basal node. The vertical axis represents time. The terminal nodes represent the taxa under investigation, and the lengths of the branches (i.e., internode distances) represent time intervals (= anagenetic periods) between cladogenetic events.



character state to another can be estimated using the Poisson distribution. For the *i*th character, the expected number of changes in the *j*th branch, given t_j, is

$$E[\, C_{ij} \,] = r_i t_j \qquad (1)$$

where r_i is the rate of change of character *i*.

The probability of a single change of character *i* on the *j* th branch, then, is

$$P_{iC} = e^{-ritj} . r_i t_j \qquad (2)$$

and the probability of no change is

$$P_{iNC} = e^{-ritj} \qquad (3)$$

The likelihood of a tree can be written as the product of the likelihoods of each character given the topology of the tree:

$$L = \prod_{i=1}^{n} L_i \qquad (4)$$

The likelihood of each character can be calculated as follows (after Felsenstein, 1981):

$$L_i = \sum_{l} \sum_{k} \prod_{j} P_{.ijkl} \qquad (5)$$

where $P_{.ijkl}$ is the probability of either "change" or "no change" of character *i* on branch *j* given a certain arrangement *k* with *l* as the ancestral state;

j is the number of branches on the tree;

k is the number of different ways that character *i* can be assigned to a tree of a given topology; and

l is the number of character states that can be assigned to HEU_0.

Figure 5.2 illustrates the likelihood of Character 1 (from Table 1) is obtained, for the topology ((AB)C). Note that, in order to solve the likelihood function, we have to estimate a number of parameters: 1) the state of character 1 in HEU_0 (the root node); and 2) the possible distribution of character changes on the branches of the tree. For this reason, ((AB)C) is compatible with at least 4 possible distributions of changes of character 1 (Figures 2a-d).

Clearly, as the number of characters and EUs increases, the computation of the likelihood function becomes more difficult. To overcome this, a final assumption which simplifies the likelihood function is made. This is that the probability of change for any character is such that

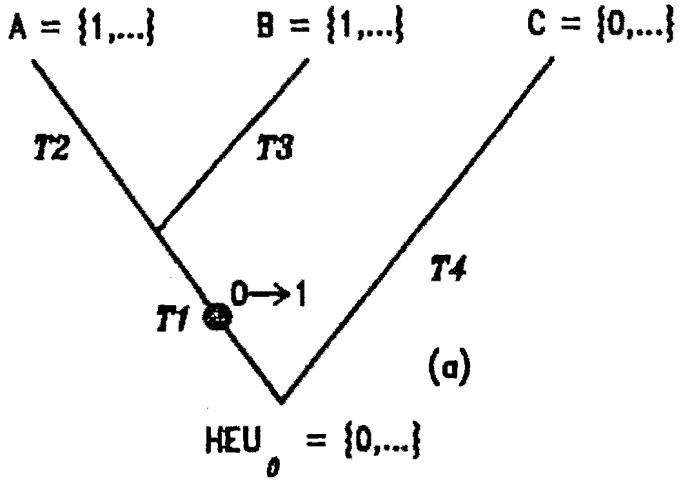
$$r_i^c \prod_{p} t_p \gg r_i^{c+1} \prod_{q} t_q \qquad (6a)$$

This states that the joint probability of *c* changes of character *i* is much greater than the joint probability of $c+1$ changes. This assumption is a more general extension of that given by Farris (1973) and Felsenstein (1978, 1981); and in order for it to be met, the individual r_is must be small, and t_js not too unequal, so that they can be replaced by a constant, *t*. For the assumption to be met, the following must hold:

$$r_i \leq [(2m - 2)t\,]^{-1} \qquad (6b)$$

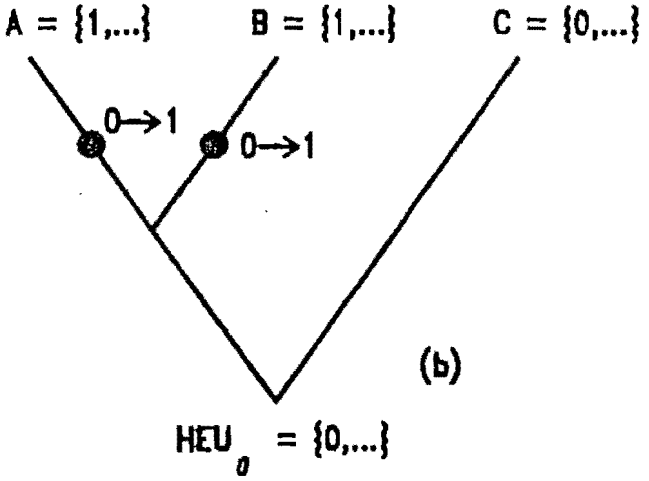
In other words, the expected number of changes of character *i* should not be greater than 1, over the

Figure 5.2 Calculation of the likelihood of character 1, from Table 5.1, given the topology ((A,B),C). The ancestral state of character 1 may be "1" (HEU_0={1,...}) or "0" (HEU_0={0,...}). Also the way the states can be arranged on the tree differs. The sum of the likelihoods for each possible arrangement and ancestral state assignment gives the total likelihood of character 1.



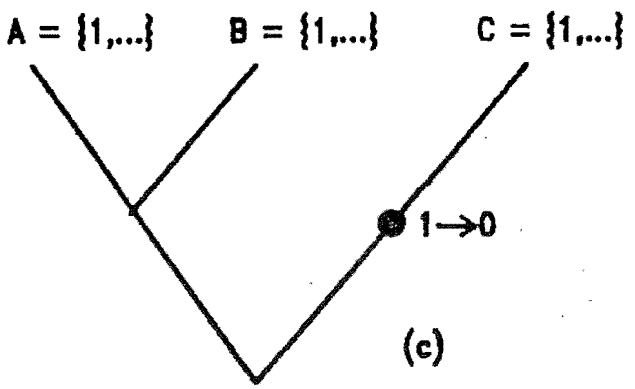
HEU_0 = {0,...}

$$L_{1(a)} = P_{1C} \times P_{1NC} \times P_{1NC} \times P_{1NC}$$



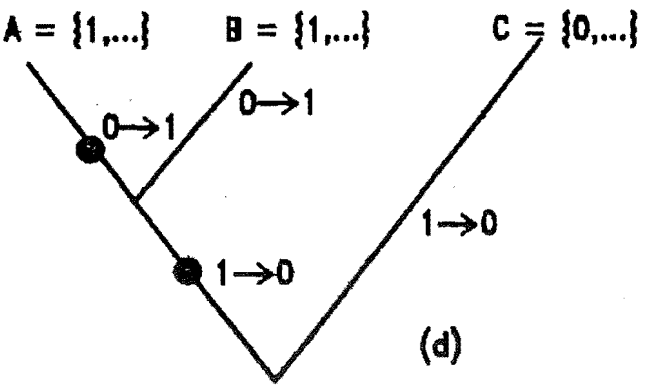
HEU_0 = {0,...}

$$L_{1(b)} = P_{1NC} \times P_{1C} \times P_{1C} \times P_{1NC}$$



HEU_0 = {1,...}

$$L_{1(c)} = P_{1NC} \times P_{1NC} \times P_{1NC} \times P_{1C}$$



HEU_0 = {1,...}

$$L_{1(d)} = P_{1C} \times P_{1C} \times P_{1C} \times P_{1C}$$

Calculation of likelihood function for Character 1 under topology ((A,B),C):

$$L_1 = \sum_{k=0 \text{ or } 1} \sum_{k=1}^{4} \prod_{j=1}^{3} P_{.1jkl}$$

$$L_1 = L_{1(a)} + L_{1(b)} + L_{1(c)} + L_{1(d)}$$

whole tree.

The inequality given at (6a) allows us to remove from the likelihood function all terms that contribute very little to its outcome. For each character, this includes discounting a) any arrangement of character changes that requires more than the minimum number of steps; and b) the assignment of character states to HEU_0 which "force" more than a minimum number of character state changes. In short, (6a) allows us to remove the summation signs from (5), so that the likelihood function for the *i*th character is now

$$L_i = \prod_j P_{.ij.} \qquad (7a)$$

and for a given tree, is

$$L = \prod_i \prod_j P_{.ij.} \qquad (7b)$$

Taking logs on both sides, we get

$$\ln L = \sum_i \sum_j \ln P_{.ij.} \qquad (7c)$$

Note also that (6a) allows us to identify a tree by its (ordered) set of numbers of character changes. By applying (6a), maximising the likelihood of a topology is equivalent to finding that vector of numbers of character changes which maximises the probability of obtaining the observed data. Therefore, the emphasis has been shifted from an estimation of *topology* only, to an estimation of evolutionary events, or evolutionary *history*.

There is, in fact, a potential problem with this approach, one which Felsenstein (1978) has pointed out. In this model, there are as many parameters to be estimated as there are characters. This means that as a data set increases with the addition of more characters, more parameters must be estimated. As Felsenstein (1985) notes, the addition of "infinitely many parameters" violates one of the sufficient conditions for the consistency of an ML estimate. This does not mean that the estimate of phylogeny obtained *will not be* consistent, only that we cannot say for certain that it is. Nonetheless, as I will argue later, a sub-optimal likelihood solution is still more acceptable than one which does not make any probabilistic assumptions (e.g., parsimony), even if there is no gaurantee that it will converge to the true tree as more characters are added.

Equation (7c) may be rewritten by taking account of the number of changes of each character for a given tree. If, for instance, a character changes once, the log of its total likelihood will be

$$\ln L_i = \ln P_{iC} + (2m - 3) \ln P_{iNC} \qquad (8)$$

In general, if the *i* th character changes c_i times, its log-likelihood will be

$$\begin{aligned} \ln L_i &= c_i \ln P_{iC} + (2m - 3 - c_i) \ln P_{iNC} \\ &= c_i (\ln P_{iC} - \ln P_{iNC}) + (2m - 3) \ln P_{iNC} \qquad (9) \end{aligned}$$

The log-likelihood of the tree, taking all characters into consideration, will be

$$\ln L = \sum_i c_i (\ln P_{iC} - \ln P_{iNC}) + (2m - 3) \sum_i \ln P_{iNC} \qquad (10)$$

Substituting (2) and (3) into (10),

$$\ln L = \sum_i c_i \ln r_i t + (2m - 3) \sum_i r_i t \qquad (11)$$

Since $(2m - 3) \sum_i r_i t$ is constant for all trees, the term can be eliminated from (11) to give

$$\ln L = \sum_i c_i \ln r_i t \qquad (12)$$

Therefore, (12) has to be maximised to obtain the ML estimate of phylogeny. Note that under Condition (6b), equation (12) is always negative.

Figure 5.3 illustrates the principles of obtaining the estimate: given rates of character change in a set S, a number of trees may be generated, each with its characteristic vector of character change. Some of these trees cannot give the observed data, and are discounted. Of those that can, the tree with the highest probability of occurring is chosen.

Note, however, that maximising (12) however requires that we have some idea of the rate of change for each character. Often, such information is unavailable, particularly when dealing with morphological data. There are two main ways of dealing with this problem. First, an estimate of the r_is may be derived using extrinsic and *a priori* information (see Chapter 4) or by iterative techniques. These methods are described in more detail in the next section of this chapter. The second strategy is to compute the "total" likelihood of a particular tree, under a range of r_is. This method will also be described in a later section of the chapter.

METHODS WHICH PROVIDE APPROXIMATIONS TO M-L ESTIMATES

In the following discussion, I will show how different procedures currently available for developing phylogenetic hypotheses can be interpreted within the maximum-likelihood framework. The procedures can be divided into three categories:

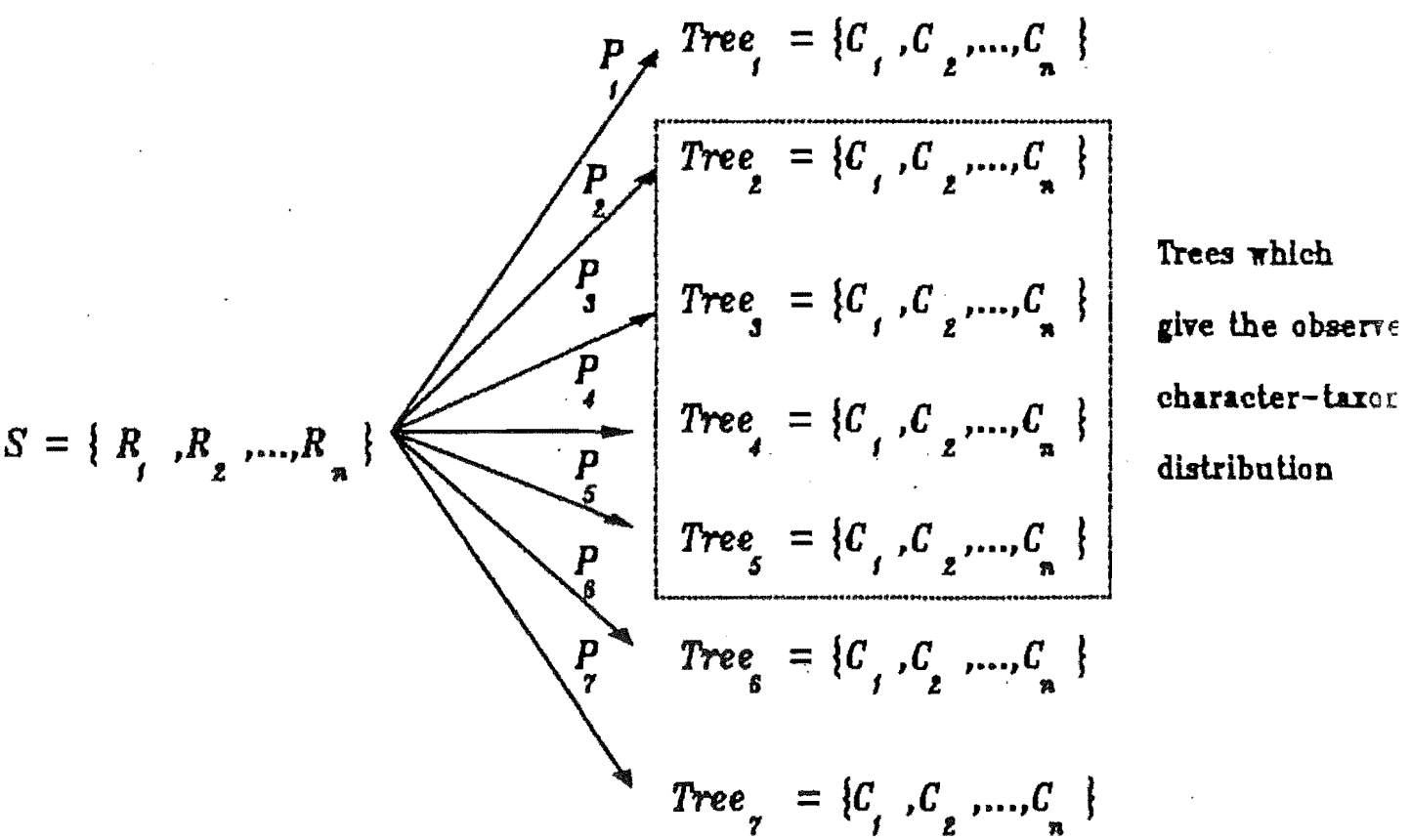
1. *Commonly used methods;* these include Parsimony and Compatibility Analysis, as well as In-group and other methods for polarising characters.
2. *Weighting methods (a priori and a posteriori);* for example, Farris's (1971) successive approximations approach, and the use of character compatibilities to estimate rates of character change.
3. *A posteriori optimality criteria;* two measures, the Optimal Character Consistency Index and the Optimum-Likelihood Index (Chapter 3) are considered.

1. Commonly used methods

Parsimony

The maximum parsimony or minimum-steps method retrieves a tree in which the total number of character state changes is minimised (Camin and Sokal, 1965; Kluge and Farris, 1969). It is quite

Figure 5.3 Principle of obtaining the Maximum Likelihood Estimate of an evolutionary tree. Given a set, S, of rates of character change, a series of trees (Tree1 - Tree7) can be generated, each represented by a vector of hypothesised character changes, $\{C_1,C_2,...,C_n\}$ and each with its own probability, P_1 - P_7, of occurring. Only some of these trees will give the observed character-taxon distribution of observed (in the diagram, these trees are surrounded by the dashed lines). Of these, the tree with the highest probability is chosen as the ML estimate of evolutionary history.



easy to see under what conditions the procedure of parsimony or minimum-steps evolution will maximise (12) and give a M-L estimate of an evolutionary tree. In the simplest case, when the minimum-length tree has no conflicts of character assignments (i.e., when its consistency index is 1), it is equivalent to the ML tree. This is intuitively obvious: since our model is subject to the constraint that two character state changes are far less probable than one, any tree in which all characters change only once must be the most likely tree.

Similarly, if the probability of a change is equal for all characters, then the term ($\ln r_i t$) will be constant for all i, and the likelihood maximum will depend only on the sum of the number of changes over all characters. Because ($\ln r_i t$) is negative, in order to *maximise* the likelihood function, one would have to *minimise* the number of character changes.

Therefore, if one can be reasonably sure that the probabilities of change for all characters are not significantly different, then trees generated using parsimony will also be ML trees. When we have no prior knowledge about the state of our taxonomic data, with respect to rates of change, it is still valid to use parsimony as an exploratory tool, or a first approximation.

Compatibility Analysis

Suppose that in a taxonomic survey of a group of organisms, two subsets of taxonomic characters appear to be present: the first consists of characters with very low rates of change, whereas those in the second subset have high rates of change, such that ($\ln r_i t$) [for all characters of this set] is much smaller. To minimise (12), one must ascribe the minimum number of changes to characters with very low rates of change. This is because the values of ($\ln r_i t$) for these characters will be very negative, and will have the effect of lowering the value of the likelihood.

However, we rarely have sufficient information to identify which characters have this property. The safest method of estimating the phylogeny of the group is to find all trees with the largest number of characters that are hypothesised to have arisen only once. This is the basis of compatibility analysis: the search for the largest *clique* of non-conflicting and uniquely derived characters.If only one such tree can be constructed using these characters, then it is likely, within the constraints of the model, to be the M-L estimator of phylogeny. If there are a number of such trees, then the M-L estimator will be one of them. It is important to note here that there is no need to postulate equal rates of change for compatibility analysis. Furthermore, if no two characters are fully covariant (i.e., are present and absent in the same groups of EUs), then the size of the largest clique must be in the range between (m-2) and 2(m-1) in order to obtain a fully bifurcating tree. If the proportion of "random" characters is high, however, it is unlikely that the largest clique size will equal this value. Therefore, the set of possible trees which estimates phylogeny increases rapidly as clique size decreases. Consequently, there is a corresponding decline in the utility and practical value of the results.

In-group assignment of ancestral character states

In order to conduct an efficient phylogenetic analysis, it is necessary to know the order in which the states of a particular character evolved, i.e., its polarity. When no such information is available, maximum-likelihood estimation offers a solution.

It follows readily from (6a) that we should assign character states to the HEU_0 character vector in such a way that the minimum number of changes required, given the ancestral state, is, in fact, the minimum over both possible ancestral states. Figure 5.4 illustrates how this may be done. Given the distribution of character 1, it is possible to partition the EUs into two groups: (A,B,C) and (D,E,F,G). For any topology in which the member EUs are kept in their respective groups (e.g., Figure 5.4a&b), assigning state 1 or 0 to HEU_0 results in the same number of changes on the tree. However, for other topologies (e.g., 5.4c-f), the assignment of state 1 as the ancestral state results in a minimum number of state changes taken over both character states.

Essentially, this corresponds to the in-group method of character polarisation, in which character states that are common amongst all EUs are taken to be ancestral. This "common-is-primitive" method of character polarisation can be used as an *a priori* method for determining character polarity, and as a means of rooting an unrooted evolutionary tree.

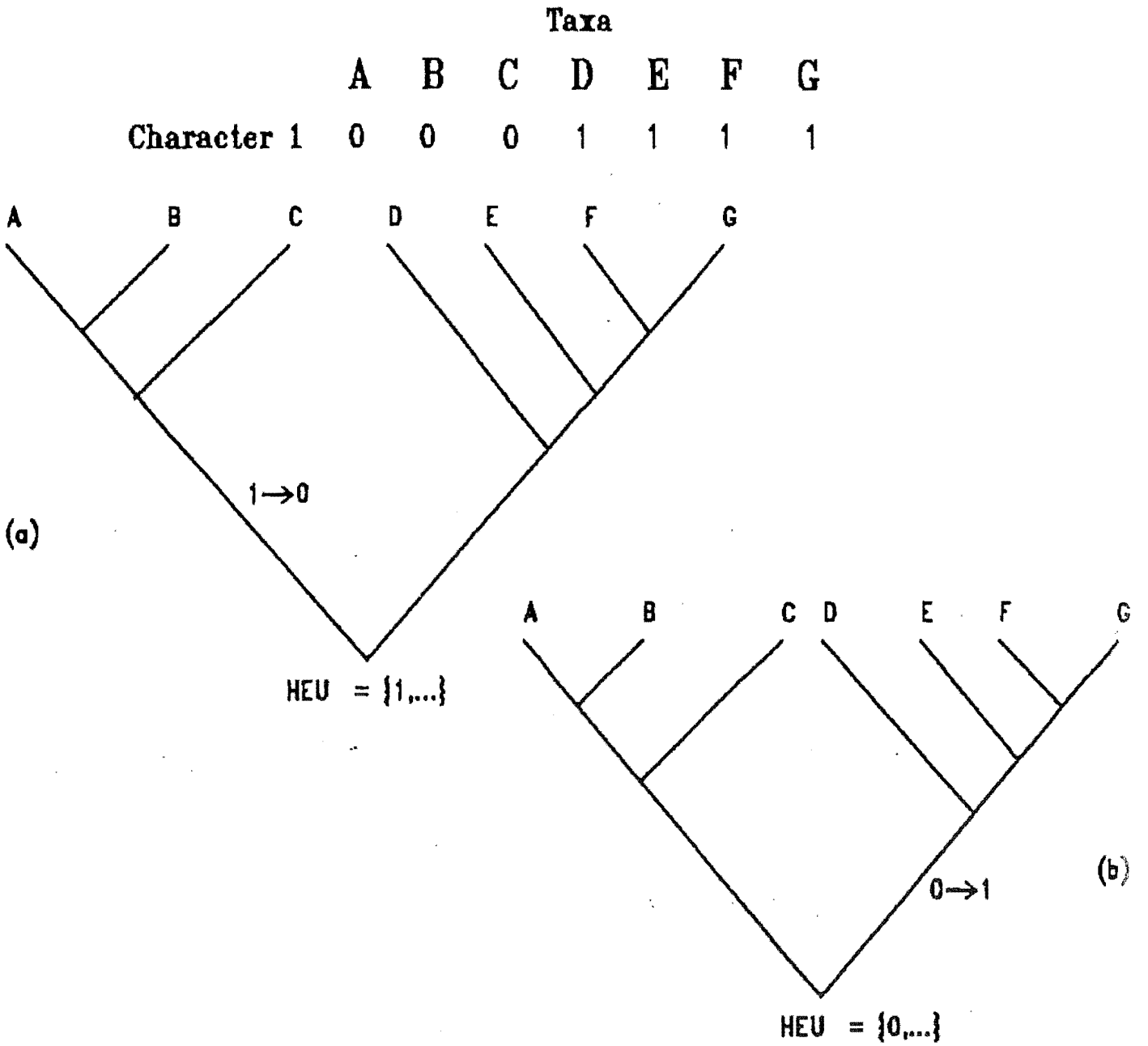
A cautionary note must be added, however: determination of character polarity is a very important part of phylogenetic reconstruction. An incorrect decision about the polarity of "good" characters can effectively turn a phylogenetic tree on its head, so that monophyletic groups become paraphyletic assemblages. While the common-is-primitive method is statistically defensible, it cannot be defended on the grounds of evolutionary theory. After all, it is possible that a feature possessed by a majority of OTUs arose as a novelty (i.e., synapomorphy) early in the evolutionary history of the group. For this reason, it is only advisable to use the in-group method as a last resort.

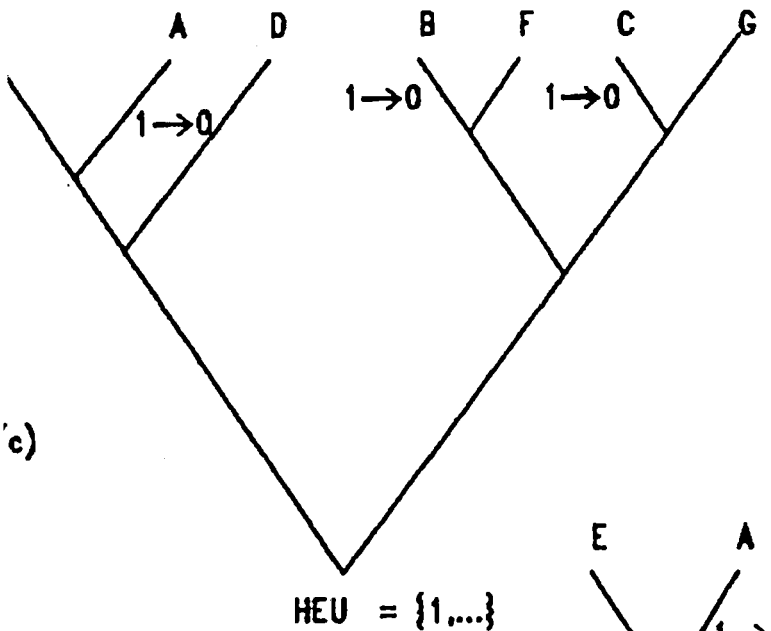
Other methods of character polarisation, such as the use of ontogenetic information (Lundberg, 1973; Nelson, 1978), and the out-group comparison method (Watrous and Wheeler, 1982), are obviously not derivable from maximum-likelihood techniques. However, they offer solutions for identifying the character state vector of HEU_0, so that fewer parameters need to be estimated. Polarity decisions made on the basis of a background knowledge of the biology of the group in question and evolutionary theory, are far more valuable for phylogenetic reconstruction, in much the same way as growth functions derived specifically from what is known about the biology of an organism are valuable.

2. Weighting methods

Commonly used methods of phylogenetic reconstruction such as parsimony and compatibility approximate maximum-likelihood solutions under restrictive conditions only. However, Equation (12) facilitates the development of methods that are not so constrained. Equation (12) can, in fact, be rewritten as:

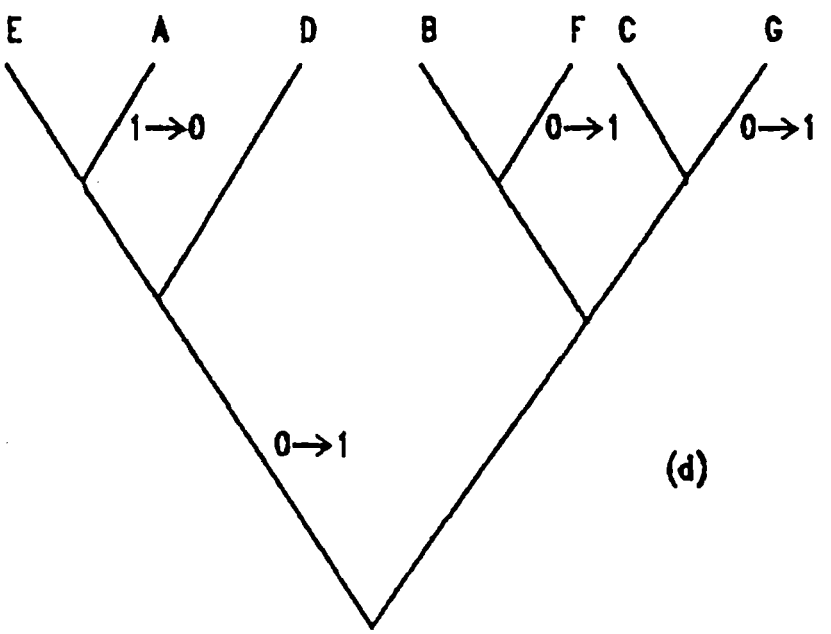
Figure 5.4 (a-f) A rationale for the ingroup assignment of character states. See text for explanation.





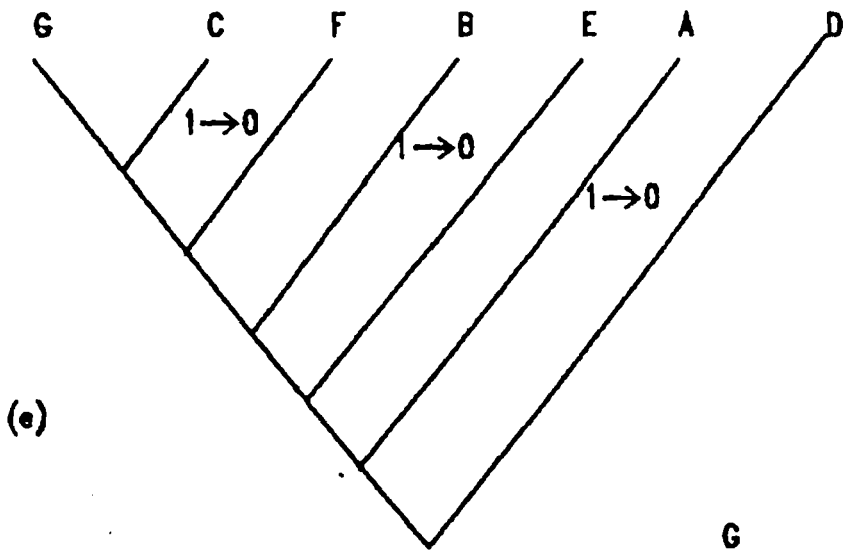
c)

HEU = {1,...}



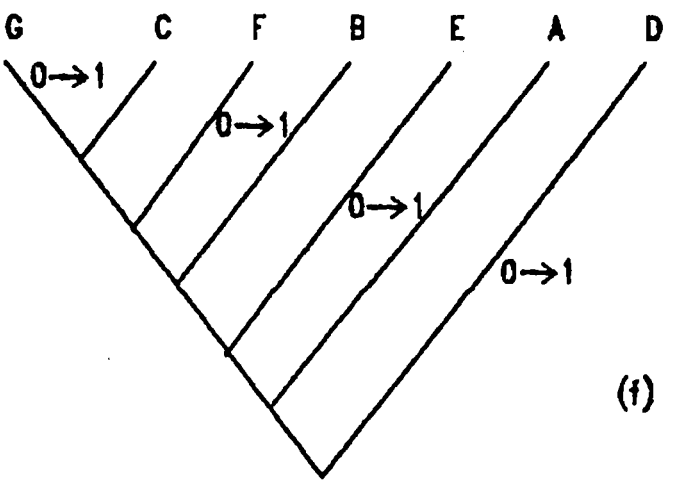
(d)

HEU = {0,...}



(e)

HEU = {1,...}



(f)

HEU ={0,...}

$$\ln L = \sum_i c_i w_i \qquad (16a)$$

where w_i is ($\ln r_i$).

Maximising $\ln L$ is equivalent to minimisng

$$-\ln L = -\sum_i c_i w_i \qquad (16b)$$

This is exactly the same as conducting a weighted parsimony analysis where the weight of the i th character is (- $\ln r_i$). This procedure was first recommended by Felsenstein (1981).

The problem with such an approach is that in order to apply the weights, a systematist must have some idea of the value of the rates of change for the characters. Since evolutionary rates of character change are rarely empirically observable (except perhaps for molecular data obtained from prokaryotes), they have to be estimated. The following methods provide ways of arriving at such estimates.

Farris's successive approximations approach

Farris (1969) developed a method of character weighting for parsimony analysis which he called a "successive approximations approach". The method involved the use of a weighting function which was estimated iteratively by using the character consistency indices from a succession of "runs" of a parsimony program on a computer. Although Farris' weighting function was not a mathematical "relative" of the likelihood function (see Chapter 4), he did show that an iterative approach to the derivation of character weights (using a variety of weighting functions) resulted in a final estimate that was closer to the true tree than the initial (most parsimonious) estimate of phylogeny.

The following procedure is a modification of Farris's original successive approximations approach only insofar as the calculation of character weights is concerned.

1. Decide on a maximum number of runs.
2. Conduct a parsimony analysis.
3. If the consistency index of the tree is 1, STOP. If the tree does not differ topologically from the previous run, STOP (this condition only applies after the first run). If the maximum number of runs have been reached, STOP.
4. Count the number of times each character changes (call this c_i).
5. Estimate the expected number of changes per branch of the i th character (i.e., r_i) as c_i / ($2m$ - 2).
6. Calculate the weight of the i th character as - $\ln c_i$ / ($2m$ - 2).
7. Repeat Step 2.

The maximum number of runs will be determined by the number of EUs, number of characters, and computing time for each run. However, Farris found (using his concave-unbounded weighting function) that for 35 EUs, no analysis exceeded 20 runs. I have found that for moderately sized data sets (15 to 25 EUs) it is usually adequate to set the upper limit at 10 runs.

Character compatibility procedures

Another way of estimating rates of character change involves the use of character compatibilities. Two characters are compatible if and only if they can be found on the same phylogenetic tree without either changing more than once. If a character is compatible with a large number of other characters, then it is likely that the number of changes of that character, averaged over all possible trees, will be low (see Chapter 3). The proportion of characters with which a particular character is compatible, therefore, is a useful indication of its rate of change.

Two (binary) characters are compatible if not every possible combination of character states is observed in the study EUs (Meacham, 1980). Conversely, if the observed states of Characters A and B are (0,0), (0,1), (1,0), and (1,1), then A and B are *incompatible*. In Table 5.2, for instance, Character 1 is incompatible with Characters 3 and 4, but compatible with Characters 2 and 5.

The rate of change of a particular character, say i, can be determined as follows. Every time a character with which i is compatible changes only once, then i will change only once. If i is compatible with all characters, then on every possible tree it will change only once. If, on the other hand, a character with which i is incompatible changes once, then i will be forced to change more than once. Felsenstein and Archie (in press; cited in Archie, 1989) showed that as the number of of characters increases, the expected number of changes of a character approaches $(3n - 2)/9$, asymptotically. However, the maximum number of times i will be forced to change cannot be greater than the number of EUs that share the derived state of i. Therefore, it is reasonable to estimate the expected number of changes of A, *over the whole tree,* as

$$E[\, C_i \,] = U_i M_i + V_i \qquad (16a)$$

and the expected rate of change per branch segment as

$$E[\, C_i \,]/(2n - 2) \qquad (16b)$$

where U_i is the proportion of characters incompatible with i;

V_i is the proportion of characters compatible with i; and

M_i = min { $[(3n-2)/9]$, number of EUs with the derived state of i} .

Again, character weights are calculated by taking the negative log of (16b). In this case, though, there is no need for an iterative estimation procedure.

Felsenstein's Threshold Weighting Procedure

Felsenstein (1981) developed a weighting procedure which included parsimony and compatibility analyses as special cases, within a likelihood framework. His threshold weighting procedure was based on the assumption that, for every character, there is a maximum number of changes which will remove a significant proportion of the probability of giving the observed data from the likelihood of a tree. Any changes beyond this threshold will affect the likelihood marginally.

Therefore, if the threshold for character i is x_i, then all things being equal, trees in which i changes $x_i, x_i+1, x_i+2,...$, are equally likely to give the observed data. In parsimony analysis, the threshold for

Table 5.2 Incompatible and compatible characters. Two characters are incompatible if in a character-taxon matrix, they appear in all possible combinations, i.e., (0,0), (0,1), (1,0), and (1,1). The compatibilities of the characters given in (A) are indicated in the pairwise compatibility matrix (B). (+) and (-) indicate compatibility and incompatibility, respectively. Note that characters which are autapomorphies (i.e., occur only in a single EU), for example, Characters 2 and 8, are always compatible with all other characters.

	characters							
	1	2	3	4	5	6	7	8
EU_1	1	1	0	0	0	1	0	0
EU_2	0	0	1	0	1	0	0	0
EU_3	1	0	1	1	0	1	0	0
EU_4	0	0	0	1	0	0	1	1
EU_5	0	0	0	1	0	0	1	0

(A)

	1	2	3	4	5	6	7	8
1	.							
2	+	.						
3	−	+	.					
4	−	+	−	.				
5	+	+	+	+	.			
6	+	+	−	−	+	.		
7	+	+	+	+	+	+	.	
8	+	+	+	+	+	+	+	.

(B)

all characters is the number of EUs under consideration. This means that every change counts. For compatibility analysis, the threshold is 2, i.e., only the first change counts and trees in which characters change two or more times are equally likely.

Despite the fact that the concept of a threshold to character change is simple, statistically sound, and intuitively appealing it is difficult to implement as a weighting procedure for the following reasons:

1. *A priori* decisions have to be made about the appropriate threshold for each character.
2. Because the threshold scheme involves the use of a stepped function (and not a smooth one), it is impossible to derive an adequate weighting function that can be applied in a parsimony analysis.
3. Since no weighting function can be applied, the only recourse is to examine all possible trees, and select those that satisfy the threshold criterion. The task becomes monumental if the number of EUs is greater than 5 . For all practical purposes, the threshold weighting function is of limited value. However, it is of theoretical importance in that it shows the link between parsimony and compatibility.

There are other weighting schemes: Farris's (1969) weighting function has already been mentioned; others include weights derived from the ratio of observed and expected incompatibilities (Hendy and Penny, 1985), and the iterative compatibility weighting derived by Moody and O'Nolan (1986). None of these are derived explicitly from a likelihood model, however, although all follow the obvious course of weighting "good" characters (i.e., those with a low rate of change) highly in contrast to "poor" characters. The results of simulation trials in which these methods were applied [e.g. those reported in Chapter 3, as well as those given in Farris (1969) and Penny and Hendy (1985)]resulted in trees which were closer approximations to the true trees, than initial and unweighted most parsimonious trees. The general rule can be stated thus:

PARSIMONY + CHARACTER WEIGHTING ≈ THE TRUE TREE

A posteriori optimality criteria

In parsimony or compatibility analysis, it often happens that there are a number of different, but equally acceptable, trees. In such situations, a systematist has the option of either using an iterative weighting function such as the one described in the previous section, or a supplementary optimality criterion to select a subset of trees. Four optimality criteria have been developed: Farris's (1972) *F*-value, the *D* measure of Brooks *et al* (1987), and my Optimal Character Consistency and Optimum-Likelihood Indices (OCCI and OLI, respectively; Chapter 3). The F-value was originally developed for continuous data and attempts to find the best match between the patristic distances of EUs, given a particular tree, and phenetic distances. Trees that minimise the difference between patristic and phenetic distances are chosen. *D* is an information-theoretic measure, and selects the tree(s)that

maximise(s) cladogenetic information. The OCCI retrieves the tree(s) for which the number of characters that change only once is maximised, whereas the OLI selects from a set of most parsimonious trees, the tree that has the highest likelihood of giving the observed data. Of the four indices, only the last is derived from a maximum likelihood function, and will be described here.

Development of the OLI is based on the assumption that a suite of most-parsimonious trees gives some idea about the number of changes one can expect for any character. Instead of estimating a single rate of change for each character, the OLI is calculated on the basis of a range of possible rates of change (expressed as the number of character changes over the whole tree). Therefore, for each character, the log-likelihood for any particular tree, T, can be calcuated as :

$$\ln L_{iT} = c_{iT} \sum_{s=1}^{N} \ln (c_{is} t)/(2m - 2) \qquad (15a)$$

where L_{iT} is the likelihood of character i of tree T;

c_{iT} is the number of changes of character i on tree T; and

N is the number of most parsimonious trees.

Equation (15a) can be re-expressed as

$$\ln L_{iT} = c_{iT} \sum_{s} \ln (c_{is}) + N.c_{iT} \ln t /(2m - 2) \qquad (15b)$$

and taking all characters into account,

$$\ln L_{.T} = \sum_{i} (c_{iT} \sum_{s} \ln c_{is}) + N \ln t /(2m - 2) \sum_{i} c_{iT} \qquad (15c)$$

Since $N \ln t/(2m - 2) \sum_{i} c_{iT}$ is constant for all members of the set of most parsimonius trees, Equation (15c) reduces to

$$\ln L_{.T} = \sum_{i} c_{iT} \sum_{s} \ln c_{is} \qquad (15d)$$

$\sum_{s=1}^{N} \ln c_{is}$ is equivalent to $\ln (\prod_{s=1}^{N} c_{is})$. For convenience, then Equation (15d) is taken as

$$\ln L_{.T} = \text{OLI} = \sum_{i} c_{iT} \ln \bar{c}_{i.} \qquad (16)$$

where $\bar{c}_{i.}$ is the geometric mean of the number of changes of character i calculated over the set of most parsimonious trees. The tree which maximises the OLI is taken as the Optimum-Likelihood estimate of phylogeny.

In 20 simulation trials in which EUs were assigned character states of random and phylogenetic characters, and multiple most parsimonious trees were found, the OLI failed to retrieve the true tree, or the closest approximation to it (in terms of the number of identical clades), only once (Chapter 3).

DISCUSSION

The use of models in evolutionary reconstruction

Biologists readily accept that mathematical models reflect reality imperfectly. This in no way detracts from their utility. For instance, the predictions of an imperfect model may be close enough to the observed data to warrant an acceptance of the model as a "working hypothesis". In the same

fashion, the use of modelling procedures (and I include statistical techniques such as ML estimation in this category) is justifiable if the models derived from the application of such procedures are close enough to "reality" or, in the absence of any direct information about what reality is like, are feasible, given our current view of reality.

Modelling procedures, such as those described above, make a number of unrealistic assumptions about the nature of the evolutionary process. However, if, after applying these procedures, the hypothesised relationships of the EUs are supported by additional independent evidence, then it is clear that the procedures should be treated as viable methods for phylogenetic reconstruction. Simulation trials [(such as those given in Chapter 3, and reported in Farris (1969) and Penny and Hendy (1985)] indicate that these and related methods perform well in comparison to standard techniques such as unweighted parsimony analysis.

Review of assumptions

1. Dichotomous evolutionary trees. By assuming that the evolutionary tree is strictly dichotomous, it is possible to hold the number of nodes and branches of a tree to a constant value of $(2m - 2)$. This in turn allows Equations (8) and (9) to be estimated simply. If the number of EUs, m, is sufficiently large, and the number of multichotomous cladogenetic events is relatively small, then failure to meet the requirement of strict dichotomy will not significantly affect the likelihood of any particular tree.

2. Independent characters. Two characters are statistically independent if and only if the expression of one character in no way influences the expression of the other. Inclusion of a set of non-independent characters is equivalent to giving these characters greater weight. This is undesirable, and most systematists try to select characters that are either known or believed to be independent. It is important to realise that characters that share the same distribution among EUs are not necessarily dependent, and need not be removed from an analysis. In essence, whether two characters are independent or not must be decided on the basis of what is known about the biology of the group in question. If there is reason to suspect non-independence, then it is possible to measure the degree of association using any of a number of indices, e.g., χ^2. [Note: It is inappropriate to use the value of χ^2 to statistically *test* the association between two characters, because their occurence in a group of EUs will be influenced additionally by the phylogeny of the group (Felsenstein, 1981). However, there is no reason why the 95%-level of a χ^2_1 cannot be used as a critical value].

3. Parallel changes and equiprobable transition rates. According to the likelihood model developed in this paper, two EUs may share the same derived state either because they each inherited the state from a common ancestor, or because the state arose independently in each EU as a result of convergent evolution. If there is good reason to believe that a derived state arose only once, then it is equivalent to assuming that the rate of change of the character in question is very low. Consequently the character should be weighted highly.

It is also assumed that both "additions" and "deletions" (or reversals) have the same probability of

occurring, although some authors have argued that this assumption may not necessarily be so (Katz, 1988). Farris (1977) has developed a model in which a derived state is allowed to arise only once, whereas multiple deletions are permitted (Dollo parsimony).

4. Equal branch lengths. The requirement for equal branch lengths (Criterion 6a) facilitates a simple and practicable solution to the likelihood function. However, in order for this criterion to be satisfied, the phylogenetic tree must be symmetrical (Fig. 5.4a). Asymmetrical phylogenetic trees have branches of different lengths (Fig. 5.4b), and the likelihood model given here will not be appropriate. Hendy and Penny (MS) have noted that a parsimony analysis conducted on taxa for which the true tree has "long edges" (i.e., unequal anagenetic periods), can converge to a wrong estimate of the tree. They recommend breaking up long edges by adding more taxa to the data set. In effect, this is equivalent to stating that in order for parsimony to work, the true tree should be symmetrical.

The consistency of the estimated tree

As stated earlier, the likelihood model developed here requires an estimation of the number of changes for each character. Therefore, as Felsenstein (1978) points out, as more characters are added the number of parameters to be estimated increases. As a result, one of the sufficient conditions for consistency is not satisfied. Felsenstein's (1973) method, which involves an estimation of branch lengths instead of character changes, requires only that a finite number of parameters (i.e., the "true" branch lengths) be estimated, *if the number of taxa remain constant.* If, however, the number of taxa increases, Felsenstein's estimator of phylogeny will not meet the sufficient condition for consistency also. With molecular data, for instance, there may be sequence information for only certain genes. The number of nucleotide bases (which serve as characters) of a particular segment of the genome are constant, but the number of taxa for which these sequences are determined may increase. Under such circumstances, Felsenstein's method may give inconsistent estimates.

However, the consistency of an estimate, while a desirable property, is not the sole consideration in the choice of a method. Another factor that should influence choice is the testability of the resultant phylogenetic hypothesis. In all the methods detailed above, the phylogenetic hypothesis that results can be tested both directly (i.e., by testing the monophyly of certain clades using information which is known to be ancestrally determined), or indirectly, by examining the hypotheses of rates of character change (see Chapter 4). Unlike parsimony and compatibility, which are simple but which result in untestable hypotheses, the techniques given here, are easily applied *and* testable. Furthermore, they often perform as well as more stringent ML procedures.

Conclusion

The heuristic approximations to ML estimators provided in this paper rely on a Likelihood Model with some restrictive constraints. These include the requirement for symmetrical trees, dichotomous branching, and independent characters. However, the value of the approach derives from the fact that characters are permitted to differ in their rates of change. By allowing this, the

methods described here are free of what is, arguably, the most troublesome problem that plagues parsimony analysis. Essentially, the methods discussed in this paper effect a workable compromise between the mathematically demanding techniques of maximum-likelihood estimation, and the simple but inefficient procedures of parsimony and compatibility.

PART III

THE PHYLOGENY AND TAXONOMY OF THE PRONOCEPHALIDAE LOOSS, 1902 (PLATYHELMINTHES : DIGENEA)

CHAPTER 6

THE PHYLOGENY OF THE PRONOCEPHALIDAE LOOSS, 1902 (PLATYHELMINTHES : DIGENEA)

INTRODUCTION

The Pronocephalidae Looss, 1901 (Platyhelminthes:Digenea) is a family of parasitic monostomes usually found in the intestinal tract of aquatic reptiles, most commonly chelonids. According to the classification given by Yamaguti (1972), the family consists of 31 genera, and about 87 species. Many genera are mono-specific, and many species have been erected on the basis of a few individuals from a single host. However, the classification of the Pronocephalidae has remained remarkably stable at the generic level. With categories higher than genus, however, there has been a great deal of reshuffling and numerous revisions since 1931 (Price, 1931; Ruiz, 1946; Skrjabin, 1955; Yamaguti, 1958, 1972; Groschaft and Tenora, 1981). Yamaguti's (1972) classification is most often used as the basis for pronocephalid classification, and I will use it as an initial guide to the genera and sub-families of the Pronocephalidae. The applicability of other classifications will be discussed in Chapter 8.

Two other monostomatous families, the Notocotylidae Luhe, 1909 and the Rhabdiopoeidae Poche, 1926 are most similar to the Pronocephalidae structurally, and a phylogenetic analysis of the Digenea by Brooks *et al* (1985) suggested that the three groups formed a monophyletic assemblage of families. A feature of the Pronocephalidae that distinguishes it from the Notocotylidae and the Rhabdiopoeidae, is the presence of a muscular cephalic ridge that usually rings the anterior portion of the body dorso-ventrally, below the oral sucker. This "head collar" is found in some form or another in almost all pronocephalid species. Except for one sub-family (the Parapronocephalinae Skrjabin, 1955), the Pronocephalidae is differentiated from the Notocotylidae by the absence of ventral tegumentary papillae or glands. The position of the ovary relative to Mehlis' gland is another distinguishing feature. Thus, in the Pronocephalidae, the ovary is anterior or lateral to Mehlis' gland, whereas in the Notocotylidae, Mehlis' gland is alway posterior to the ovary. Members of the Rhabdiopoeidae differ from the pronocephalids in the structure of the excretory system with the rhabdiopoeids possessing numerous well-differentiated excretory diverticles in the posterior region of the body.

Until now, systematic studies of the Pronocephalidae have relied to a large extent on the expertise and interpretations of the taxonomist(s) concerned. However, in the last three decades a suite of techniques that enable classifications to be constructed on the basis of rigorous principles, and allow systematic analyses to be repeated by other workers, have become

available. One such technique is phylogenetic systematics, and in this chapter, I apply the methods of phylogenetic systematics to reconstruct the evolutionary history of the species of the Pronocephalidae. The weighted parsimony method described by Farris (1969) and discussed in Chapters 4 and 5, is used, and the resulting phylogenetic hypothesis forms the framework for the classification of pronocephalid genera given in the next two chapters (Chapters 7 and 8).

METHODS AND MATERIALS

A phylogenetic analysis is a two-stage process:

1. *Character analysis*, in which taxonomic characters are selected and and the different states (= phenotypic expressions) of these characters are identified; and
2. *Phylogenetic reconstruction*, in which an hypothesis of evolutionary relationships and character evolution is erected.

Each of these stages will be described in turn.

Character analysis

General Comments

In order to conduct a satisfactory phylogenetic analysis of the Pronocephalidae, it was necessary to obtain first-hand information about the species, and the characters used to classify the group. Furthermore, it was felt that morphological features that had not been used before might prove useful as taxonomic characters. Therefore, type and other specimens of as many species as were available were examined. Table 6.1 lists all specimens examined and the institution from which they were borrowed. Where specimens of species were unavailable, morphological information was obtained from original published descriptions. Taxonomic characters used in the phylogenetic analysis had to satisfy one primary criterion: they had to be monomorphic in a majority of pronocephalid species.

All characters were categorised as either *well-defined characters* or *questionable characters*. Well-defined characters a) were those that were easily differentiated into discrete character states, and could be reported accurately, b) were easily seen in specimen preparations, and c) were usually reported in published descriptions. Questionable characters failed to satisfy at least one of these requirements, and were more susceptible to coding errors due to misinterpretation, and/or incorrect reporting.

The rationale behind categorizing taxonomic characters in this manner has been discussed in Chapter 4, but it is worth restating. The *a priori* categorization of characters provides independent, albeit *circumstantial*,

Table 6.1 List of pronocephalid specimens examined.

Abbreviations:

USMNHC = United States National Museum Helminthological Collection.

NR = Naturhistoriska Riksmuseet, Stockholm.

BM = British Museum (Natural History).

QM = Queensland Museum

MN = Museum fur Naturkunde der Humboldt-Universitat zu Berlin.

Species names in parentheses indicate that these names have since been synonymised with the taxon immediately preceding them.

Species	Institution	Specimen No.	Remarks
Pleurogonius bilobus	NR	1873	Deposited by Looss.
P. laterouterus	USNMHC	73317	Paratype
P. linearis	NR	2640	
	NR	1869	Deposited by
	NR	1828	Looss
	NR	1842	
	USNMHC	73339	
P. longiusculus	NR	1835	
	NR	1854	
	NR	1867	
	NR	1868	Deposited by
	NR	1969	Looss
	NR	1978	
	NR	1964	
	NR	1830	
	NR	2635	
P. minutissimus	NR	1916	Deposited by Looss.
P. pomacanthus	USNMHC	8090	
	USNMHC	8087	
P. puertoricensis	USNMHC	73319	Paratype
P. trigonocephalus	NR	2637	Deposited by
	NR	1852	Looss
	NR	2636	
	USNMHC	73340	
P. truncatus	QM	GL12230	Co-type.
Pronocephalus obliquus	NR	1863	
	NR	1860	
	NR	1923	
	NR	2638	
	NR	1861	
	NR	1984	Deposited by
	NR	1973	Looss.
	NR	1956	
	NR	1865	
	NR	1982	
	NR	1979	
(*Monostomum trigonocephalum*)	USNMHC	35286	

Species	Institution	Specimen No.	Remarks
Diaschistorchis takahashii	USNMHC	73012	
	USNMHC	73011	
Epibathra crassa	NR	1961	
	NR	2645	
	NR	1965	
	NR	1827	Deposited by Looss.
	NR	1868	
	NR	1969	
	NR	1978	
	NR	1964	
	NR	1830	
	NR	2635	
E. stenobursata	USNMHC	73313	Holotype
Glyphicephalus latus	USNMHC	73315	Paratype
G. lobatus	NR	1817	
	NR	1750	Deposited by Looss.
	NR	2012	
	NR	2017	
	NR	1958	
	NR	1926	
	USNMHC	73330	
G. mcintoshi	USNMHC	39308	Holotype
G. solidus	NR	1972	
	NR	1970	Deposited by Looss
	NR	1975	
	NR	1924	
Iguanacola navicularis	USNMHC	43401	Deposited by Gilbert.
Macravestibulum eversum	USNMHC	55343	Paratype
M. kraatzi	USNMHC	39058	Paratype
M. obtusicaudatum	USNMHC	42038	
Metacetabulum invaginatum	USNMHC	73333	
M. karachiense	BM	1973.10.12.1-3	Paratypes
Myosaccus amblyrhynchi	USNMHC	9217	Holotype
Parapleurogonius brevicaecum	USNMHC	74052	Paratype
	BM	1979.4.10.175-225	

Species	Institution	Specimen No.	Remarks
Barisomum candidulum	USNMHC	77794	
(*Glyphicephalus candidulus*)	USNMHC	39307	
B. erubescens	USNMHC	77796	
Cetiosaccus galapagensis	USNMHC	9215	Deposited by Gilbert.
Charaxicephalus robustus	NR	1959	Deposited by Looss.
	USNMHC	9619	Autotype
Cricocephalus albus	NR	2043	
	NR	1971	
	NR	1844	
	NR	2641	
	NR	1960	Deposited by Looss.
	NR	1966	
	NR	1857	
	NR	1846	
	NR	1847	
	NR	1834	
	USNMHC	73005	
	USNMHC	8441	
C. megastomus	NR	1974	Deposited by Looss.
	NR	1851	
	NR	1866	
	USNMHC	73006	
	USNMHC	73329	
C. resectus	NR	1929	Deposited by Looss.
	NR	1897	
	USNMHC	73007	
Desmogonius desmogonius	USNMHC	73007	
	USNMHC	73006	
	USNMHC	68232	
Diaschistorchis gastricus	BM	1954.9.14.403	
D. multitesticularis	USNMHC	39495	Paratypes
	BM	1911.7.111-3	Paratypes
	MN	6440a-c	Paratypes
D. pandus	QM	GL11166	Deposited by Johnston.
	USNMHC	73332	
Diaschistorchis spp.	Dr. D. Blair's personal collection: approx. 70 mounted and unmounted/fixed specimens.		

Species	Institution	Specimen No.	Remarks
Pyelosomum cochlear	NR	1921	
	NR	1986a	Deposited by
	NR	1986b	Looss.
	NR	1885	
	NR	2639	
	NR	1859	
	USNMHC	9665	Autotype
P. longicaecum	USNMHC	8910	Type
P. parvum	QM	GL12232	Type
P. posterorchis	USNMHC	73331	
P. renicapite	USNMHC	74860	
(*Astrorchis renicapite*)	USNMHC	51965	
(*Monostomum sphargidis*)	USNMHC	8094	Co-type
Renigonius cuorensis	USNMHC	73059	Paratypes

evidence by which we can qualitatively assign a "confidence value" to the resultant phylogenetic hypothesis, particularly an hypothesis pertaining to character change. For instance, there is no reason to question the results of a phylogenetic analysis in which the characters hypothesised to have changed only once (i.e., consistent characters) are also those that have been labelled as "well-defined" *a priori*. However, a phylogeny in which consistent characters are primarily those that were judged to be questionable must be treated with suspicion, because of the comparatively high risk of errors. Such an hypothesis would be given a low "confidence value".

Descriptions of the characters used in the analysis is given below. The numeric codes assigned to their states, and where necessary, a discussion of any points requiring elaboration, e.g., terminology, and taxonomic utility, are also provided. The letters "W" or "Q" in parentheses indicate whether the characters are well-defined or questionable, respectively. Unless stated otherwise, no assumptions have been made about the pattern of evolutionary change of characters with more than two states (i.e., they have been treated as unordered characters).

Taxonomic Characters

1. *Dorsal cephalic ridge absent=0; dorsal cephalic ridge present=1.* (Q). One of the components of the pronocephalid head collar, the dorsal cephalic ridge, is a muscular elevation just below the level of the oral sucker. It is absent in some genera (e.g., *Pseudobarisomum* Siddiqi and Cable,1960, *Desmogonious* Stephens, 1911, *Rameshwarotrema* Lakshman Rao, 1975, *Cetiosaccus* Gilbert, 1936, *Metacetabulum* Freitas and Lent, 1938) and weakly developed in others (e.g., *Renigonius* Mehra, 1939). Whether the absence of the dorsal ridge is a secondary reduction, or a state shared with members of the Notocotylidae and Rhabdopoeidae, which lack a dorsal ridge, is unknown.

2. *No ventral cephalic modifications = 0; Lateral muscular folds present=1; ventral ridge present, but incised=2; ventral ridge present and complete=3; ventral ridge absent, but muscular bundle below oral sucker present=4.* (Q). Figure 6.1 shows the different modifications of the ventral cephalic region of pronocephalids. The presence of a muscular bundle below the oral sucker is a unique feature of the genus *Parapronocephalum* Belpolskaia, 1952, and as Sinclair (1972) points out, is very different from the ventral ridge of pronocephalid species in general. In addition, Linton (1910) has stated that for the species *Barisomum candidulum* (Linton) Price, 1931, the ventral head collar is variable and can take the form of a bilateral thickening and fold (Fig 6.1d) or possess a deeply incised ventral ridge (Fig. 6.1c). Similarly,

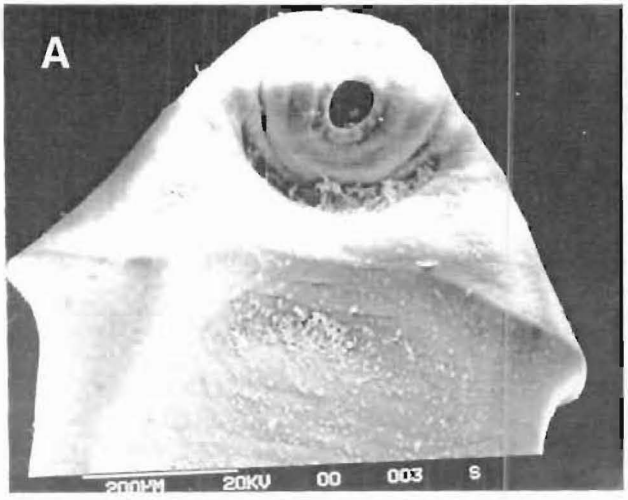
Figure 6.1 Types of pronocephalid head collars as seen under the scanning electron microscope.

A. *Charaxicephalus* sp.: Ventral ridge complete and unincised.

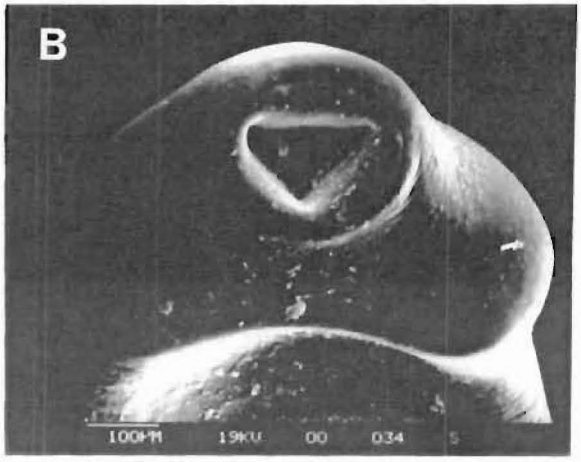
B. *Cricocephalus* sp.: Shallow ventral incision.

C. *Glyphicephalus* sp.: Deep ventral incision with lateral margins appearing lobe-like.

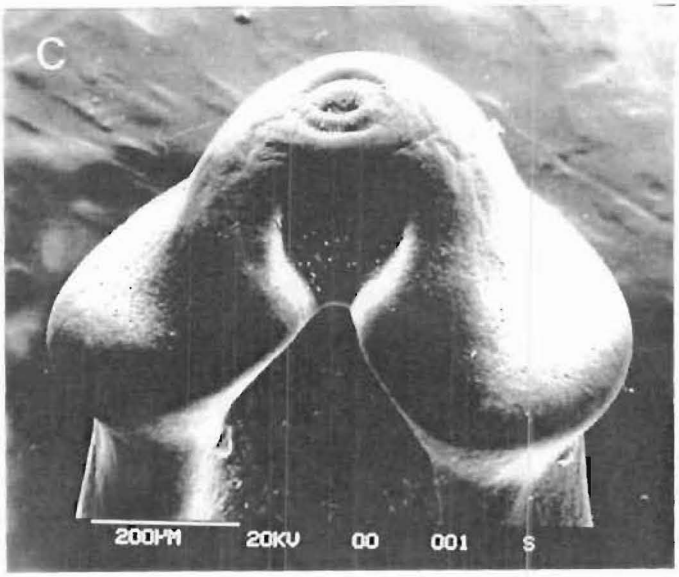
D. *Pleurogonius* sp.: No ventral ridge, but with lateral margins thickened and folded.



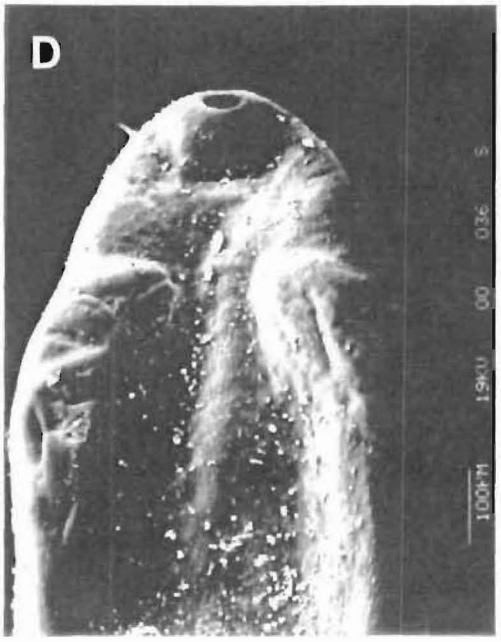
CHARAXICEPHALUS SP.



CRICOCEPHALUS SP.



GLYPHICEPHALUS SP.



PLEUROGONIUS SP.

Jahan (1970) has found that the species *Neopronocephalus triangularis* has variable ventral cephalic morphology. Furthermore, descriptions of ventral cephalic morphology are often incomplete in the taxonomic literature, stating only that the head collar is strongly or weakly developed.

3. *Oral sucker terminal=0; Oral sucker sub-terminal=1.*(Q).

Depending on the degree to which the animals have been pressed during fixing, there will be distortion of the relative positions of certain morphological structures, particularly those at the ends or sides of the body. The oral sucker is susceptible to such distortion.

4. *Diameter of oral sucker less than 25 percent body width of animal=0; oral sucker between 25 and 50 percent body width=1; oral sucker greater than 50 percent body width=2.* (Q). Because of the continuous nature of this character, cutoff points are arbitrary to an extent, and are meant to indicate "small", "medium", and "large".

5. *Body elongate or elliptical=0; body vermiform=1.* (W).

6. *Posterior macropapillae absent=0; posterior macropapillae dome-shaped, setose or asetose=1; posterior macropapillae conical, muscular, and invaginated=3.* (Q).

The presence of papillate structures at the latero-posterior margins of some pronocephalid genera has been noted since Looss's (1899) descriptions of the genus *Cricocephalus* Looss, 1899. However, most taxonomists have not distinguished between the different types of macropapillae. An electron microscope study of specimens obtained by D. Blair from Badu Island off North Australia, revealed that the posterior macropapillae of different genera are structurally different and of constant form within a genus (Fig. 6.2). The simplest form of macropapillae is a dome shaped structure which may or may not be setose (Fig. 6.2d and 6.2e). The genus *Charaxicephalus* Looss, 1901 possesses macropapillae of the type shown in Fig 6.2c and Fig 6.3. The papillae are invaginated, and under the light microscope, the pocket of an invaginated papilla can often be seen to abut the caecal terminus or diverticulum. It should be noted that one of the features used to distinguish the genus *Charaxicephaloides* Groschaft and Tenora, 1978, a genus that is morphologically very similar to *Charaxicephalus*, is the presence of caecal pores located in the same region of the macropapillae. I believe that these "pores" are probably nonexistent, and are artifacts caused by the close proximity of caecae and the invaginated pockets of the posterior papillae.

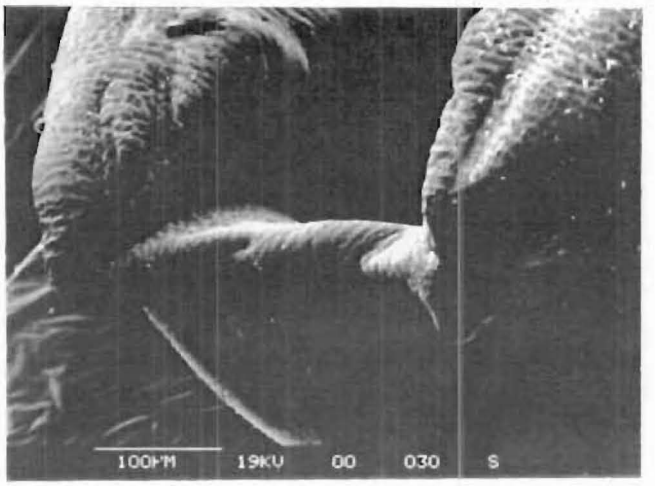
The posterior macropapillae are interesting for another reason. In the two pronocephalid species whose life histories have been

Figure 6.2 Types of pronocephalid posterior macropapillae, as seen under the scanning electron microscope.

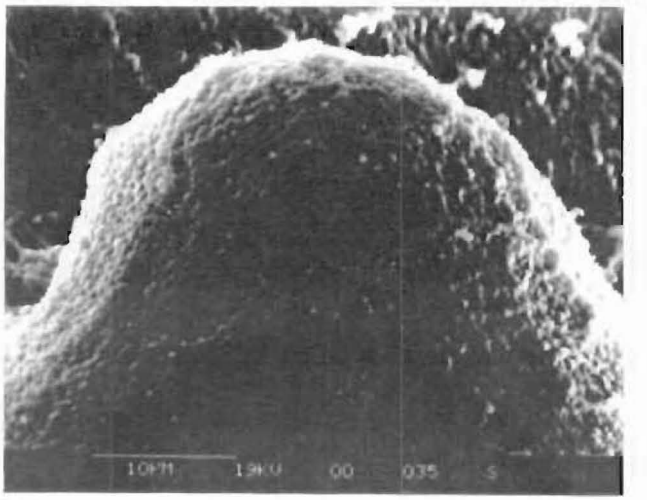
A & B) *Cricocephalus resectus*: posterior macropapillae dome-shaped, with "bubbly" tegument.

C) Invaginated, conical macropapillae of *Charaxicephalus* sp.

D & E) Setose and dome-shaped macropapillae of *Cricocephalus* sp.

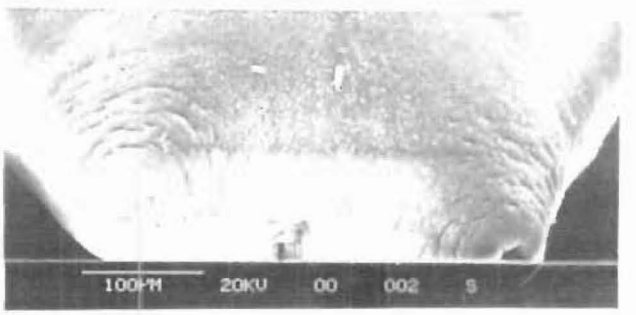


A



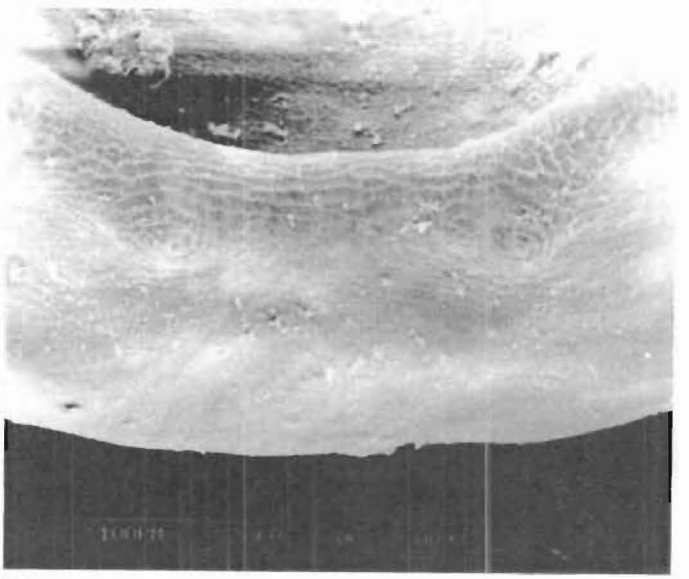
B

CRICOCEPHALUS RESECTUS

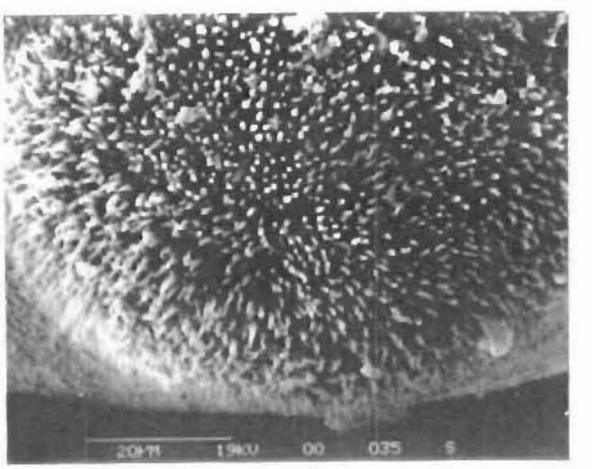


C

CHARAXICEPHALUS SP.



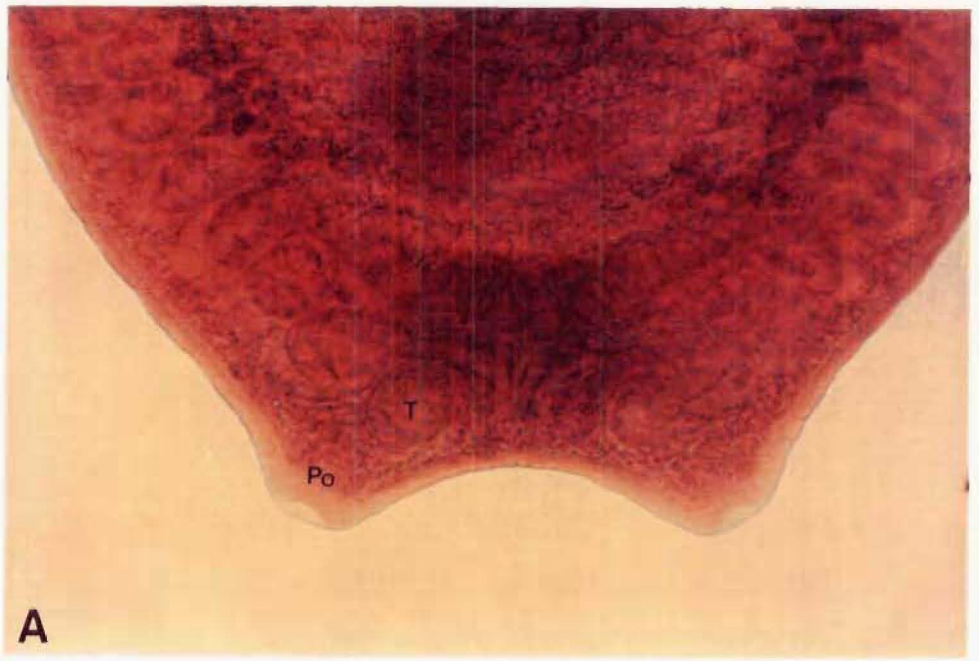
D



E

CRICOCEPHALUS SP.

Figure 6.3 View of the posterior macropapillae of *Charaxicephalus* sp. under the light microscope. (A) The invaginated pocket (Po) lies close to the caecal terminus (T). This is seen less clearly in (B).



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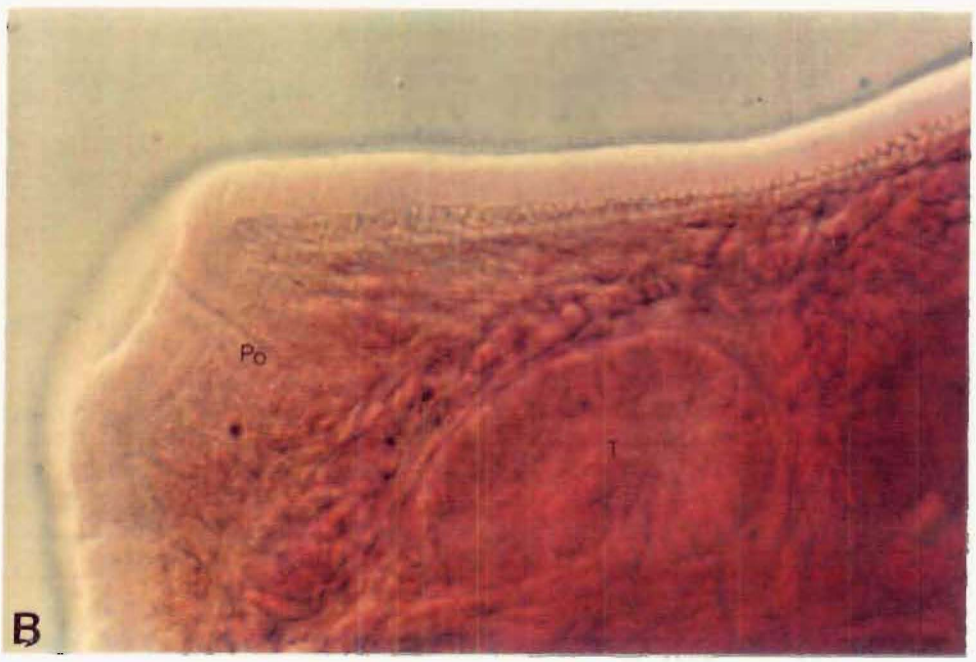

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investigated, the cercariae possessed highly motile and invaginated papillate processes in the same location as the macropapillae of some adults (Horsfall, 1930 who studied the cercariae of *Macravestibulum obtusicaudatum* Mackim, 1930; and Thapar, 1968 and Saxena, 1977, who studied cercariae of *Neopronocephalus* spp.). However, the adults of *M. obtusicaudatum* and *Neopronocephalus* spp. do not possess posterior macropapillae. It is also interesting that Porter (1936) in her drawing of *Cercaria fulvocata* Cawston, 1919 indicates the presence of these papillate processes. The adult stage of *C. fulvocata* is thought to be a notocotylid species. This supports the suggestion that the two families are closely related.

The detection of posterior macropapillae may be hindered by the tendency of pronocephalids to have the margins of the body curving ventrally. If worms are mounted in this position, it is very difficult to locate the papillae, particularly if one is not looking for them. Furthermore, there is evidence that the papillae become less prominent as the animals become older. For instance, the papillae are clearly visible in whole mounts of immature adults of *Diaschistorchis multitesticularis* Rohde, 1962 (Specimens No BM/1979.4.10.232), but absent in mature adults (Specimen No. BM/1911.7.11.1-3).

7. *Ventral papillae or ridges present=0; ventral papillae or ridges absent=1.* (W).

8. *Oesophagus short (caecal bifurcation in first quarter of body)=0; oesophagus long (caecal bifurcation in second quarter of body=1.* (Q).

The length of oesophagus is often omitted from published descriptions, and the illustrations of type specimens have been used as sources of information. Consequently, there is often no information about the variability of this character.

9. *Caeca with smooth margins=0; caeca with crenulated margins=1; caeca diverticulate, with diverticula extending to lateral margins=2; caeca diverticulate, with diverticula not extending to lateral margins=3.* (W).

10. *Caeca extend to posterior margin of body=0; caeca do not extend into fourth quarter of body=2; caeca extend to anterior margin of testes=3.* (W).

11. *Testes caecal or extracaecal=0; testes intercaecal=1.* (W).

12. *Excretory vessels not easily seen=0; excretory vessels easily seen=1.* (Q).

The visible components of the excretory system of pronocephalids consist of an excretory vesicle or bladder, and either a pair of excretory vessels which empty into the vesicle or bladder, or a system of anastomosing vessels. Although the extent to which these vessels can be seen is partially a

function of the staining and fixing technique, there are some genera in which these vessels are prominent and sometimes readily visible even in living specimens (e.g., *Pyelosomum* Looss, 1899, *Cetiosaccus* and *Metacetabulum*). In most instances, however, the vessels are only weakly visible. In the absence of information to the contrary, it is possible that the prominence of the excretory vessel may indicate monophyly. However, published descriptions often fail to note whether the excretory vesicles are well-developed or not.

13. *Excretory vessels simple or diverticulate, with no anastomoses=0; excretory anastomoses present=1.* (Q).

Although the nature of the excretory system may be a valuable taxonomic character, it is often difficult to determine its structure (J. Pearson, pers.comm.). Sometimes, excretory deposits within the tubules may outline the form of the system (as in *Epibathra crassa* Looss, 1901). However, particularly in the second and third fourths of the body, the excretory system is often is obscured by other structures such as the uterine coils and vitellaria, or is not visible because of the stain and fixing technique used.

14. *Excretory vessels meeting simply at anterior portion of body=0; vessels meeting in a network=1; vessels looping before joining anteriorly=2; vessels not meeting anteriorly=3.* (W).

Although the excretory system may not be visible within most of the body, it is usually apparent in the region of the head collar or just below it. Here the excretory vessels terminate, either uniting above the caecal bifurcation, or ending blindly.

15. *Excretory vessels identical in diameter=0; excretory vessels differ in in diameter=1.* (W).

In two genera, *Cetiosaccus* and *Metacetabulum*, the posterior portion of the excretory vessels are asymmetrical with one limb having a larger diameter than the other. No other genus has this feature.

16. *Excretory pore in posterior, dorsal and sub-terminal=0; excretory pore posterior, terminal=1.* (W).

17. *Testes symmetrical=0; testes oblique=1; testes tandem=2.* (W).

18. *Ovary in median position=0; ovary sub-median=1.* (W).

19. *Ovary anterior to or at level with Mehlis' gland=1; ovary posterior to Mehlis' gland=2* (W).

20. *Vitellaria in two compact fields=0; vitellaria in linear fields beginning pre-equatorially or at the equator=1; vitellaria in linear fields beginning post-equatorially=2; vitellaria in linear fields beginning post-equatorially, but acini arranged in bunches=3.* (W).

21. *Mehlis' gland in median position=1; Mehlis' gland sub-median=2.* (W).

22. *Uterine coils completely intercaecal=0; uterine coils extending ventral to caeca=1; uterine coils extending beyond caeca=2.* (Q).

23. *Uterine coils in transverse loops=0; uterine coils not arranged in orderly transverse loops=1.* (W).

24. *Metraterm slender=0; metraterm well developed and muscular=1.* (Q).

Although this character should be considered "well-defined", it is often omitted from published descriptions. The metraterm forms the anterior portion of the uterus and is sometimes thin-walled and poorly developed, appearing simply to be an extension of the uterus. In other cases, the metraterm is muscular with thick walls that are often folded (e.g., *Metacetabulum karachiense* Bilqees, 1974).

25. *Metraterm placed longitudinally=0; metraterm curved diagonally=1; metraterm placed transversely=2.* (Q).

26. *Metraterm shorter than cirrus sac=0; metraterm as long as cirrus sac=1.* (Q).

27. *Ejaculatory duct with thick muscular wall=0; Ejaculatory duct slender=1; ejaculatory duct absent=2.* (W).

Use of the terms "ejaculatory duct" and "cirrus" is not consistent in pronocephalid taxonomy. In this paper, the ejaculatory duct is considered to be that part of the terminal male genitalia enclosed in the cirrus sac, above the glandular pars prostatica or prostate. The cirrus is the eversible part of the ejaculatory duct. However, the cirrus is not often visible in whole mounts, because it is usually not everted.

28. *Cirrus complex placed longitudinally=0; cirrus complex curved diagonally=1; cirrus complex placed transversely=2.* (Q).

29. *Pars prostatica muscular=0; pars prostatica slender=1.* (W).

30. *Cirrus sac with constriction separating prostatic complex from ejaculatory duct=0; cirrus sac without constriction=1.* (W).

31. *Terminal portion of seminal vesicle enclosed in cirrus sac=0; terminal portion of seminal vesicle not enclosed in cirrus sac=1.* (W).

When the terminal portion of the seminal vesicle is enclosed within the cirrus sac, it is often referred to as an *internal seminal vesicle.*

32. *Cirrus complex long (one-fifth length of body)=0; cirrus complex of medium length or short (less than one-fifth body length)=1.*(Q).

33. *Male and female genital pores separate=0; common male and female genital pore, or both pores opening into a genital atrium with an external opening=1.* (Q).

This character is often unstated in published descriptions. Furthermore, in

whole mounts it is difficult to ascertain whether there is a single common opening or genital atrium, as both male and female terminal genitalia usually lie close together.

34. *Genital pores intracaecal and sub-median=0; genital pores intracaecal and median=1; genital pores caecal or extracaecal and below the level of the caecal bifurcation=2; genital pores extracaecal and above the level of the caecal bifucation=4.*(W).

35. *Eggs unioperculate=0;eggs bioperculate=1;eggs non-operculate=2.*(Q). Often unstated in published descriptions.

36. *Eggs with polar filaments=0; eggs without polar filaments=1.* (Q). The presence or absence of polar filaments on eggs, as well as the number of such filaments has been used as a feature for separating species. However, this character is subject to misidentification for a number of reasons. First, as Mehra (1932) points out, it is sometimes difficult to see polar filaments on eggs unless the eggs are extruded from the uterus. Second, eggs at the posterior end of the uterus lack filaments, whereas those at the anterior region of uterus may possess them (Coil and Reid, 1965; Bhatnagar and Gupta, 1981). Thirdly, a close examination of the eggs of one of Looss's specimens (Specimen No. NR/2642: *Epibathra crassa*) reveals that what looks superficially like a single long filament actually has a braided structure, suggesting that it consists of a number of filaments plaited together (Fig. 6.4).

37. *Body length between 1 and 5 mm=0; body length greater than 5 mm=1; body length less than 1 mm=2.* (Q).

38. *Terminal portion of ejaculatory duct without accessory vesicles=0; terminal portion of ejaculatory duct with accessory vesicles=1.*(W). Species of *Macravestibulum* possess an accessory vesicle on either side of the ejaculatory duct. When the cirrus is everted, the openings of the vesicles are seen to be sited on a pair of papillae placed lateral to the cirrus (Damian, 1961). It is interesting to note that in the type specimen of *Cetiosaccus galapagenis* Gilbert, 1936, there appears to be a papilla with a duct beside a dome-shaped cirrus (Fig.6.5). It is likely that this structure is homologous with the accessory vesicle of *Macravestibulum*, and that in the type specimen of *C. galapagensis*, the other papilla is obscured by the cirrus.

39. *Caeca parallel to lateral margins of body for most of the length of the animal=0; caeca sinusoidal=1.* (W).

40. *Ventral glands in regular longitudinal fields=0; ventral glands not arranged in order=1; ventral glands absent=2.* (W).

The following characters have been coded using a modification of the

Figure 6.4 An egg of *Epibathra crassa*. One of the filaments appears braided suggesting that it is not a single filament but rather, is composed of a number of filaments.

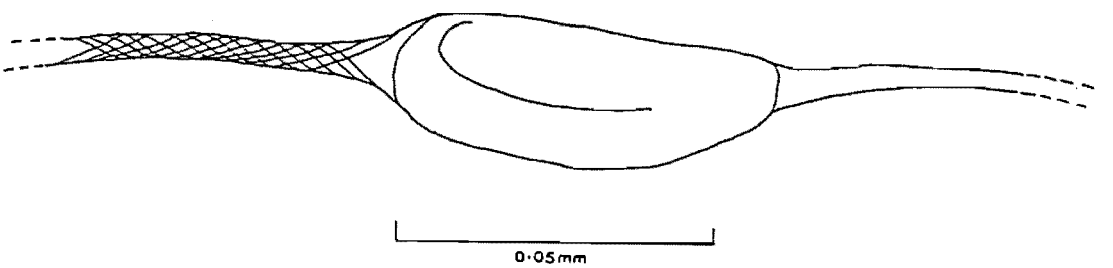
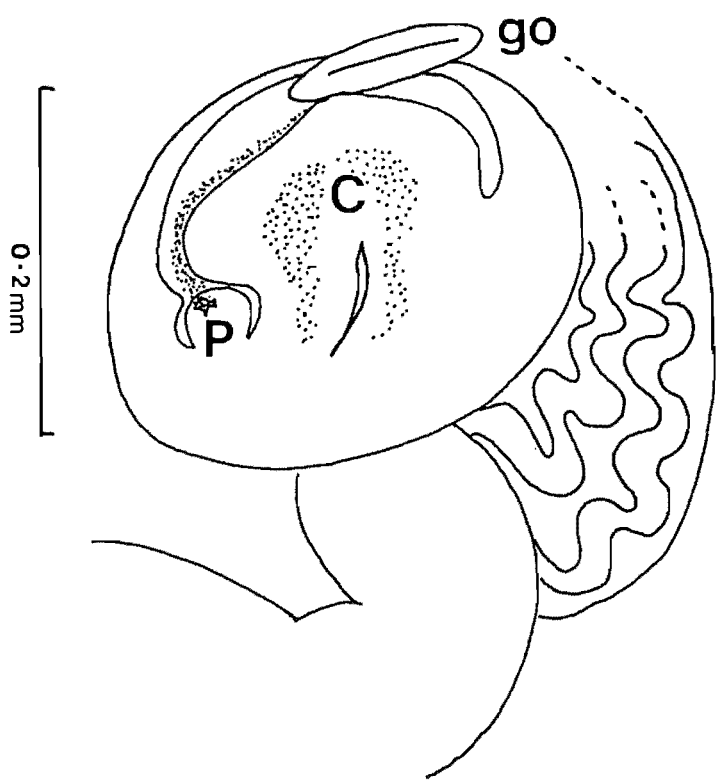


Figure 6.5 Illustration of a presumptive accessory vesicle lying next to the cirrus of *Cetiosaccus galapagensis*. The cirrus is a broad papillate structure (C), and what appears as a smaller papilla (P) with a duct lies lateral to the cirrus.



method of Non-Redundant Coding described by O'Grady and Deets (1987). This method allows hypotheses of character evolution to be incorporated into a phylogenetic analysis, and involves coding different character states in order to to reflect a putative hypothesis of character evolution depicted by a *character evolutionary tree.* The coding involves generating a number of "false" or pseudo-characters to represent each true character. Character states that are believed to have arisen from the same ancestral state are identified by sharing the same psuedostate in at least one of these pseudocharacters. I refer to characters states which share a common ancestral state not shared by other states as an *homological group.* Character states of a homological group are said to be *closely homologous.*

In a phylogenetic analysis, the pseudocharacters are treated like other characters. Therefore, taxa that have different character states that are believed to be closely homologous, have a "predisposition" towards forming a monophyletic group in the phylogenetic analysis. If two taxa have the same pseudocharacter state, and if the taxa do not appear as a monophyletic group, an additional step is added to the phylogenetic tree.

In this study, assigning some character states a value of "9" is equivalent to specifying that information about that character is missing or unknown. When "9" appears in pseudocharacters, it means that the homological relationship of the character state (represented by the codes of the pseudocharacters) with other characters cannot be hypothesised. It is left up to the phylogenetic analysis to provide an hypothesis of evolution for such character states.

41-43. *Excretory vesicle present, simple, vestibule absent, excretory vessels in Y-shaped pattern = 110; excretory vesicle present, simple, vestibule absent, excretory vessels in V-shaped pattern = 120; excretory vesicle present, digitiform, vestibule absent, excretory vessels in V-shaped pattern = 130; excretory bladder present, vestibule present = 201; excretory bladder small, vestibule present = 901; excretory bladder present, vestibule absent = 300.* (W).

In this study, the term "excretory vesicle" is used to describe the small sac into which the two excretory vessels empty. The term "excretory bladder" refers to a large, well-developed structure that is very obvious in whole mounts. Both vesicle and bladder generally open to the exterior by way of the excretory pore. Occasionally, the excretory pore empties into a vestibule, or secondary bladder as it is sometimes called. Ontogenetic evidence suggests that the excretory vesicle, bladder and vestibule are homologous structures (Kuntz, 1951). However, the precise nature of the relationship between these

structures is unknown. For example, the vestibular cavity, which is present in *Macravestibulum* has been described as a secondary bladder, situated posterior to the primary bladder (Mackim, 1930). However, ontogenetic evidence indicates that in cercariae of *Macravestibulum* spp. the vestibule arises from the same region of the excretory system as the excretory vesicles of other pronocephalid and notocotylid species do (Kuntz, 1951). In this analysis, I have treated the presence of an excretory vesicle, bladder, or vestibule as three separate states.

It should be noted that previously, only species of *Macravestibulum* were reported to have the vestibule, just posterior to the (primary) excretory bladder. The excretory bladder of *Macravestibulum* consists of a large bifurcated sac, each bifurcation proceeding as excretory vessels (Fig 6.6). However, Agarwal and Premvati (1977) and Saxena (1977), both illustrate structures that are clearly vestibules in immature adults of *Neopronocephalus* species (Fig. 6.7a&b). In his study of the cercaria of *Neopronocephalus* spp., it is surprising that Saxena (1977) did not compare this structure with the vestibule in *Macravestibulum*, because in his diagram (reproduced in Fig 6.7b) it is almost identical with that shown in the cercaria of *Macravestibulum* (Fig. 8.8a) by Horsfall (1930). The only apparent difference is that in the latter, the vestibule is eversible.

In adults, the only difference seems to be that the excretory vessels of *Neopronocephalus* empty directly into the vestibule, whereas in *Macravestibulum* an excretory bladder is present.

The putative character evolutionary tree is given in Fig. 6.8. The coding reflects the my view that the presence of a vestibular cavity is strong indication of monophyly, as is the presence of a simple excretory vesicle. However, no assumption is made about the origin of the excretory bladder.

44-50. *Testes whole, with smooth margins, mainly postovarian=1100000; testes whole, lobed, mainly postovarian=1000000; testes whole, with smooth margins, preovarian=1010001; testes lobed, pre-ovarian=1000001; testes follicular, testicular fields converging mediad, beginning at level of ovary=3001000; testes follicular, testicular fields converging mediad, beginning at level above ovary but extending below ovary=3001100; testes follicular, testicular fields not converging, beginning at level above but extending below ovary=3002900; testes follicular, testicular fields not converging, follicles completely preovarian=3009910.* (W).

The evolutionary tree for the character is given in Fig. 6.9. The two homological character-state groups represent variants of "whole testes" and

Figure 6.6 *Macravestibulum kraatzi* Damian, 1961 (after Damian, 1961), showing the vestibular cavity (V).

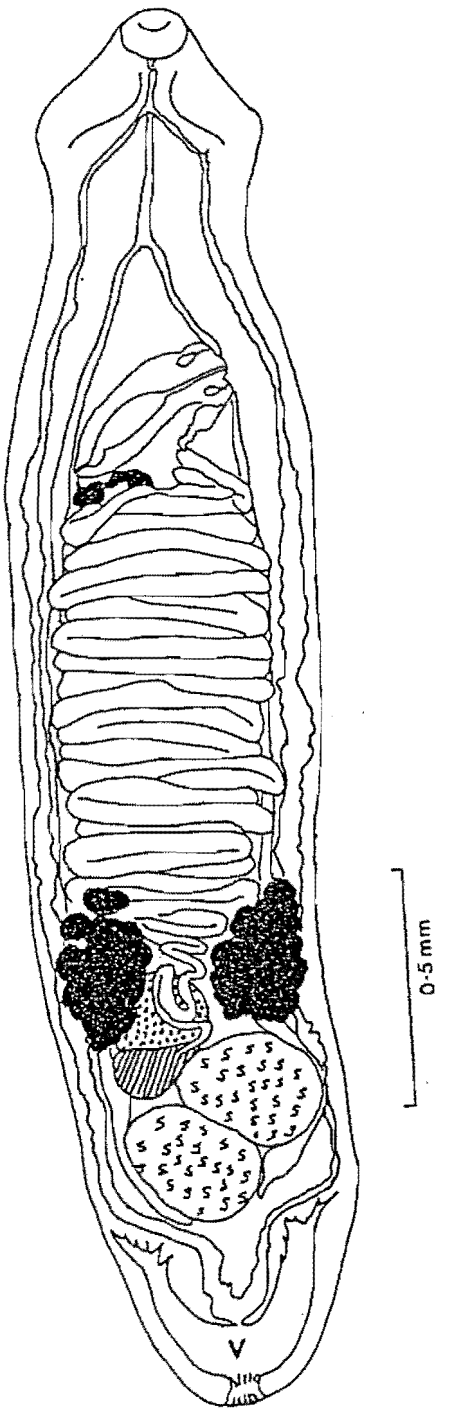


Figure 6.7 Immature adults of *Neopronocephalus* Mehra, 1932, showing the vestibular cavity. (A) After Agarwal and Premvati, 1977. (B) After Saxena, 1977.

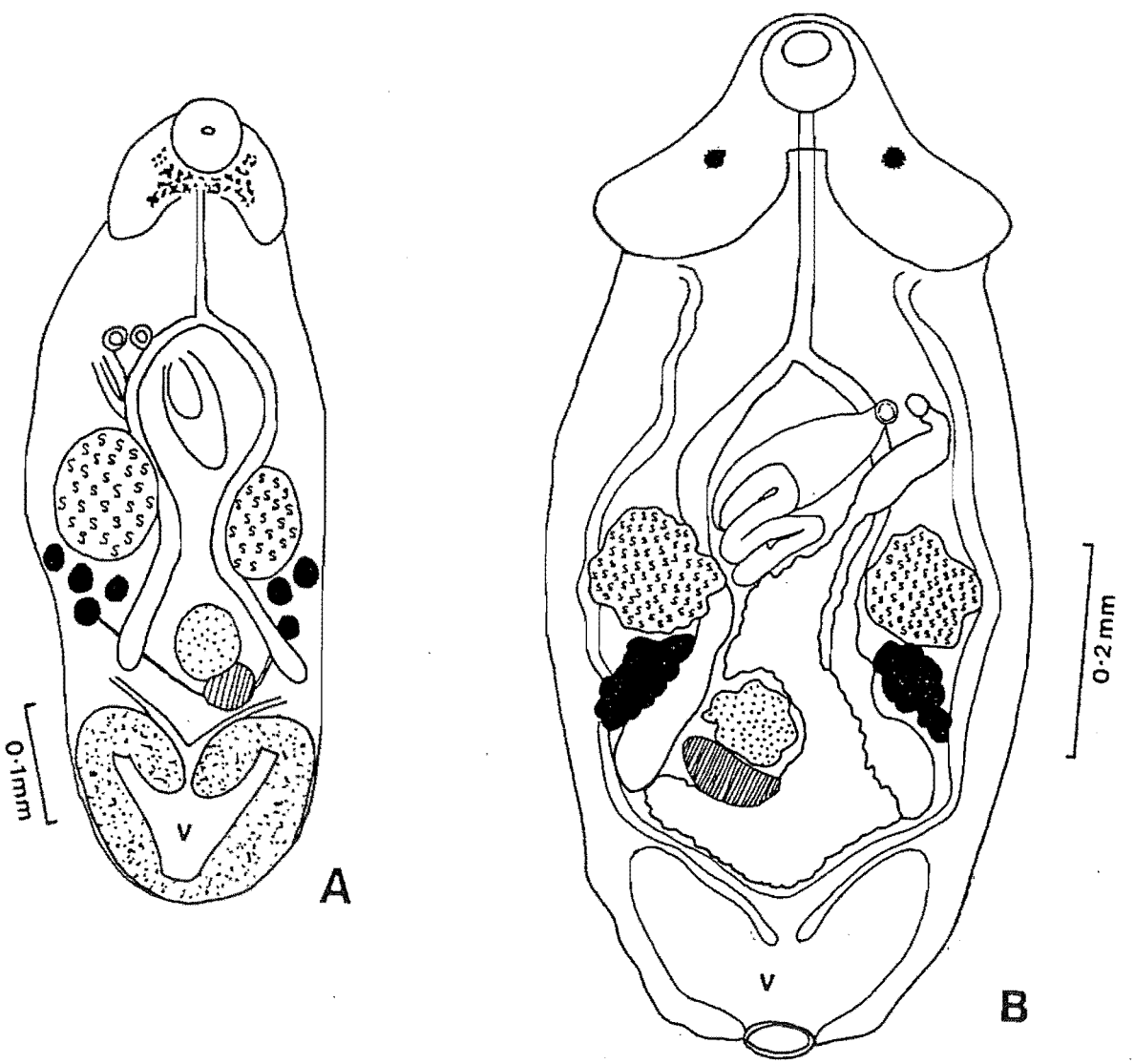


Figure 6.8 Putative character evolutionary tree for pronocephalid excretory system.

A = excretory vesicle simple, excretory vessels Y-shaped.

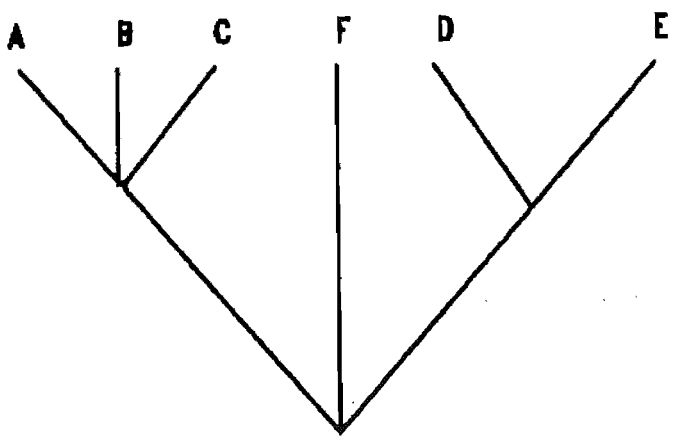
B = excretory vesicle simple, excretory vessels V-shaped.

C = excretory vesicle digitiform, excretory vessels V-shaped.

D = excretory bladder well-developed, and vestibule present.

E = excretory bladder small, and vestibule present.

F = excretory bladder voluminous, vestibule absent.



	Characters		
	63	64	65
A	1	1	0
B	1	2	0
C	1	3	0
D	2	0	1
E	9	0	1
F	3	0	0

Figure 6.9 Putative character evolutionary tree for testes structure and arrangment.

A = testes whole, with smooth margins, mainly postovarian.

B = testes whole, lobed, mainly postovarian.

C = testes whole, smooth, preovarian.

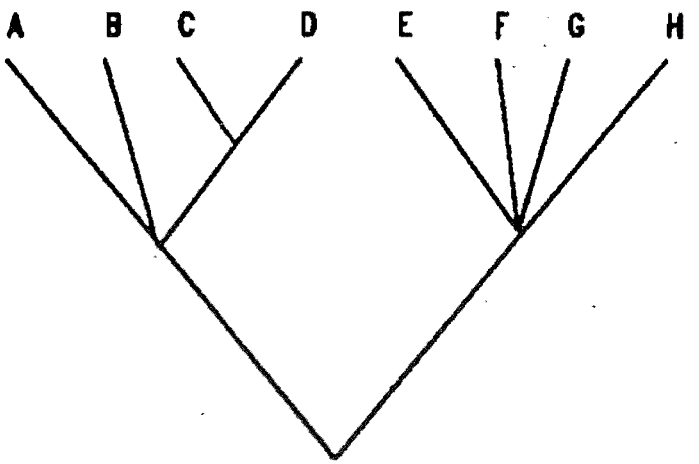
D = testes whole, lobed, preovarian.

E = testes follicular, testicular fields postovarian and converging mediad.

F = testes follicular, testicular fields largely postovarian but beginning in preovarian region, converging mediad.

G = testes follicular, testicular fields postovarian, but not converging.

H = testes follicular, testicular fields preovarian.



	Characters						
	67						73
A	1	1	0	0	0	0	0
B	1	0	0	0	0	0	0
C	1	0	1	0	0	0	1
D	1	0	0	0	0	0	1
E	3	0	0	1	0	0	0
F	3	0	0	1	1	0	0
G	3	0	0	2	9	0	0
H	3	0	0	9	9	1	0

"follicular testes". Within the "whole testes" group, another homological group of character states represent "preovarian testes".

Within the "follicular testes" group, the tetrafurcation of character states is a reflection of my unwillingness to hypothesis about the possible character evolutionary pathway for these states.

Character states for all taxa included in this analysis were identified and transcribed in a character-taxon matrix (Table 6.2). As stated above, missing information about the state of any character in a particular taxon was given the code "9". The following species of Pronocephalidae were not included in the analysis, for the reasons indicated:

Diaschistorchis multitesticularis Rohde, 1962: morphologically identical to *D. takahashii* Fukui and Ogata, 1936, except for the possession of more testicular follicles than in *D. takahashii.* Such a large number of follicles is unique to *D. multitesticularis*, and the exclusion of the species does not affect the analysis.

D. mannarensis Lakshman Rao, 1975: structurally identical to *D. prafullai* Chattopadhyaya, 1972.

Neopronocephalus gangeticus Mehra, 1932; *N. mehrai* Chatterji, 1936; *N. rotundus* Siddiqi, 1965: considered by Jahan (1970) to be synonyms of *N. triangularis* Mehra, 1932.

N. kachugai Jahan, 1970: morphologically identical to *N. orientalis* Brooks and Palmieri, 1979, except that *N. kachugai* has a seminal receptacle. Since this feature is unique to *N. kachugai*, the species may be left out of the analysis.

Choanophorus rovirosai Caballero, 1942: insufficient morphological information in published description. Since *C. rovirosai* possessed modifications to the excretory system which seemed superficially to resemble those of *Macravestibulum* and *Metacetabulum*, and this information was not available I felt that it was better to leave the genus out rather than risk using incorrect data.

The following species were omitted because sufficient taxonomic information was unavailable:

Ruicephalus minutus (Ruiz, 1946) Skrjabin, 1955.

Pleurogonius longibursatus Vigueras, 1955.

In addition to including pronocephalid species in the analysis, representative members of the Notocotylidae were also included. This was done because it is possible that notocotylids do not form a monophyletic family, but are a clade of the Pronocephalidae (D. Blair, pers. comm.). A phylogenetic analysis would suggest whether this is the case.

Table 6.2 The character-taxon matrix of the species of Pronocephalidae. The code "9" indicates missing information.

TAXA	CHARACTERS 1	10	20	30	40	50
Notocotylus spp	0 0 0 0 1 0 0 0 0 0 1 9 1 0 0 0 0 0 2 2 0 0 0 0 0 1 1 0 0 1 0 0 1 1 0 0 9 0 0 0 1 9 0 1 0 0 0 0 0 0
Rhabdiopoeius spp (outgroup)	0 0 0 0 1 0 1 0 0 0 5 9 0 9 0 0 0 0 1 2 0 0 2 1 0 0 1 1 0 1 1 0 0 1 4 0 0 1 0 0 2 1 9 0 1 0 0 0 0 0 0
Paramonostomum spp	0 0 1 0 1 0 1 0 0 0 1 9 1 0 0 0 0 0 2 2 0 0 0 1 0 1 1 0 0 1 1 0 1 1 0 0 9 0 0 2 1 9 0 1 0 0 0 0 0 0
Neopronocephalus triangularis	1 2 1 0 1 1 1 0 0 2 1 9 0 9 0 9 0 1 9 0 9 2 1 1 0 0 0 1 0 1 1 0 0 2 0 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
N. gangeticus	1 9 1 0 1 1 1 0 0 2 1 9 0 9 0 9 0 1 9 0 9 2 1 1 0 0 0 1 0 1 1 0 0 2 0 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
N. mehrai	1 2 9 0 1 0 1 1 0 2 1 9 0 9 0 9 0 1 9 0 9 2 1 1 2 0 9 1 0 1 1 9 0 0 0 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
N. ocellata	1 2 0 0 1 0 1 1 1 2 1 9 0 9 0 9 0 1 1 0 1 2 1 1 0 0 0 1 0 1 9 1 0 2 0 9 0 0 0 2 9 0 1 1 0 0 0 0 0 1
N. orientalis	1 2 0 0 1 1 1 0 0 2 1 9 0 9 0 0 0 1 9 0 9 2 1 1 0 0 2 1 0 2 0 0 9 2 2 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
N. spinosa	1 2 1 0 1 0 1 0 0 2 1 9 0 9 0 1 0 1 1 0 1 2 1 0 0 0 0 1 0 1 9 1 0 2 0 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
N. wamani	1 2 0 0 1 0 1 0 0 2 1 9 0 9 0 0 0 0 1 1 0 0 2 1 1 0 0 2 1 0 1 9 0 0 2 0 9 0 0 0 2 9 0 1 1 0 1 0 0 0 1
M. eversum	1 2 1 1 1 0 1 0 0 2 2 1 0 9 0 1 1 0 9 0 9 0 9 9 9 9 9 0 0 1 9 0 1 0 0 1 0 9 0 2 2 0 1 1 1 0 0 0 0 0
M. kepneri	1 9 0 1 1 0 1 0 0 2 2 1 0 0 0 1 1 0 1 2 0 0 0 0 0 0 0 1 0 1 1 1 0 0 0 0 0 1 0 2 2 0 1 1 1 0 0 0 0 0
M. obtusicaudatum	1 2 1 1 1 1 1 0 0 2 2 1 0 0 0 1 1 0 1 0 0 0 0 9 9 0 9 0 0 1 1 0 1 0 0 0 0 1 0 2 2 0 1 1 1 0 0 0 0 0
M. kraatzi	1 2 1 1 1 0 1 0 0 2 2 1 0 0 0 1 1 0 1 0 0 0 0 9 9 0 9 0 0 1 0 0 1 0 0 0 0 1 0 2 2 0 1 1 1 0 0 0 0 0
Cetiosaccus galapagensis	1 2 1 0 2 0 1 0 1 2 2 1 0 2 1 1 2 0 1 0 0 0 1 1 0 0 1 0 1 0 9 1 1 0 0 0 1 1 0 2 3 0 0 1 1 0 0 0 0 0
Metacetabulum invaginatum	0 0 0 0 2 0 1 0 0 2 2 1 0 1 1 1 2 1 1 0 1 0 0 0 2 1 9 0 0 1 9 0 0 2 1 1 0 0 0 2 3 0 0 1 1 0 0 0 0 0
M. yamagutii	0 0 0 0 2 0 1 0 0 2 2 1 0 1 1 1 2 0 1 2 9 9 0 1 0 0 0 0 0 1 9 0 0 2 0 1 1 0 0 2 3 0 0 1 1 0 0 0 0 0
M. karachiense	0 0 0 0 2 0 1 0 0 2 2 1 0 1 1 1 2 0 1 2 0 2 0 1 9 0 0 0 0 1 1 0 1 1 0 1 1 0 0 2 3 0 0 1 1 0 0 0 0 0
Parapronocephalum reversum	0 4 1 2 1 9 0 0 3 0 1 9 9 9 0 0 0 0 2 2 0 1 0 9 0 9 9 9 9 9 9 9 9 1 0 0 9 0 0 0 1 9 0 1 0 0 0 0 0 0
Pleurogonius puertoricensis	0 2 0 0 1 0 1 0 0 0 1 9 0 0 0 0 0 1 1 2 1 1 0 1 0 0 0 0 0 1 1 0 0 2 0 0 1 0 0 2 1 2 0 1 0 0 0 0 0 0
P. sindhi	1 2 1 0 1 0 1 0 1 0 1 9 0 3 0 0 0 1 1 2 1 0 0 0 1 0 2 1 1 1 9 1 0 0 0 1 0 0 0 2 1 1 0 1 0 0 0 0 0 0
P. trigonocephalus	0 1 0 0 1 0 1 0 1 0 1 0 9 0 0 0 0 1 1 1 0 0 0 1 1 0 0 2 0 1 1 0 1 0 0 1 0 0 0 2 1 1 0 1 0 0 0 0 0 0
P. truncatus	9 2 0 1 1 1 1 0 1 0 1 0 9 9 0 0 0 1 1 2 0 1 0 0 0 0 1 0 0 1 1 0 9 0 0 9 0 0 0 2 1 2 0 1 0 0 0 0 0 0
Pseudobarisomum holocanthi	0 0 0 0 1 0 1 0 3 0 1 1 0 0 0 0 0 1 1 0 0 1 1 9 1 0 0 1 0 1 9 0 9 2 0 9 9 0 0 2 1 2 0 1 1 0 0 0 0 0
P. ozakii	1 2 0 0 1 0 1 0 0 0 1 9 0 0 0 0 0 0 1 1 1 0 1 0 1 0 0 0 0 0 0 1 9 0 9 0 0 9 1 0 0 2 1 9 0 1 0 0 0 0 0 0
Rameshwarotrema uterocrescens	9 9 0 0 1 0 1 0 0 0 1 0 9 9 9 1 0 1 1 0 0 2 0 9 9 9 1 0 1 1 0 9 9 1 0 1 0 0 0 2 1 2 0 1 1 0 0 0 0 0
R. chelonei	1 3 0 0 1 0 1 0 0 0 1 0 9 9 9 1 0 1 1 0 0 2 9 9 9 9 1 0 1 1 9 1 9 1 0 1 0 0 0 2 1 2 0 1 0 0 0 0 0 0
Renigonius orientalis	1 3 1 0 1 0 1 0 1 9 1 9 9 9 9 0 0 1 1 2 0 0 0 0 0 1 1 0 1 1 1 1 1 9 4 0 9 0 0 0 2 1 9 0 1 0 0 0 0 0 0
R. cuorensis	1 3 0 1 1 0 1 0 0 3 9 1 0 0 0 0 0 1 1 1 0 0 0 0 0 1 1 1 1 1 1 0 1 9 4 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0

TAXA	CHARACTERS 1 10 20 30 40 50
Adenogaster spp	0 2 0 0 1 0 0 0 1 0 1 0 9 0 0 0 0 1 1 1 0 2 0 1 0 1 1 0 0 1 1 0 1 2 0 0 0 0 0 0 1 2 0 1 0 0 0 0 0 0
Adenogaster serialis	1 1 0 0 1 0 0 0 1 0 1 0 9 9 0 0 0 1 1 1 0 0 0 1 1 0 1 0 0 1 9 0 1 0 0 1 9 0 0 0 1 1 0 1 0 0 0 0 0 0
Barisomum erubescens	0 1 1 1 1 0 1 0 3 0 1 9 0 1 0 0 0 1 1 0 0 2 0 1 2 0 0 2 0 1 1 1 1 2 0 0 0 0 9 2 1 2 0 1 0 0 0 0 0 1
B. candidulum	1 2 0 0 1 0 1 0 1 0 1 1 0 0 0 0 0 9 1 2 0 1 0 1 0 0 0 0 0 1 1 0 1 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
B. mcintoshi	1 2 0 0 1 0 1 0 1 9 9 0 9 9 0 0 0 1 1 2 0 2 0 9 2 9 0 2 0 1 1 0 9 2 0 0 0 0 0 2 1 9 0 1 0 0 0 0 0 0
Raogaster indica	1 2 0 0 1 0 0 0 9 0 1 9 9 9 9 0 0 1 1 1 0 0 0 9 9 9 9 1 0 1 0 1 9 2 0 1 1 0 0 1 1 1 0 1 0 0 0 0 0 0
Epibathra stenobursata	1 2 0 0 1 0 1 0 1 0 1 0 0 3 0 0 0 1 1 2 0 2 0 1 0 0 1 0 0 1 1 0 0 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Glyphicephalus latus	1 2 1 1 1 0 1 0 0 0 1 9 0 3 0 0 0 1 1 1 0 1 0 1 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 2 1 2 0 1 0 0 0 0 0
Neocricocephalus vitallani	1 3 1 2 1 0 1 0 1 0 1 1 0 9 0 1 0 1 1 0 0 2 1 1 0 1 1 0 0 1 1 0 1 2 0 1 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Parapleurogonius brevicaecum	1 1 0 1 1 0 1 0 0 3 1 1 0 0 0 0 0 1 1 2 0 0 0 1 0 0 0 0 0 0 1 0 0 0 2 0 0 0 0 0 2 1 2 0 1 0 0 0 0 0
Pleurogonius longiusculus	0 1 1 0 1 0 1 0 1 0 1 1 0 0 0 0 0 1 1 2 0 0 0 1 0 1 1 0 0 1 1 0 1 0 0 0 1 0 0 2 1 1 0 1 0 0 0 0 0 0
P. bilobus	0 1 0 1 1 0 1 0 3 0 1 0 9 9 9 0 0 1 1 2 0 0 0 1 0 1 1 0 0 1 1 0 1 0 0 0 1 0 0 2 1 2 0 1 0 0 0 0 0 0
Cortinasoma	1 2 1 1 1 0 0 0 0 0 1 0 9 9 0 0 0 1 1 0 1 0 1 1 1 0 1 1 0 1 9 9 1 2 0 0 0 0 0 1 1 2 0 1 0 0 0 0 0 0
Pleurogonius grocotti	0 1 0 0 1 0 1 0 1 0 1 0 9 9 0 0 0 0 0 1 2 0 0 0 0 0 1 1 0 9 1 1 0 9 1 0 9 1 0 0 2 1 2 0 1 0 0 0 0 0
P. karachii	0 1 0 0 1 0 1 0 3 0 1 0 9 9 9 0 0 1 1 2 0 0 0 0 0 0 2 1 1 1 1 1 1 0 0 1 0 0 0 2 1 2 0 1 0 0 0 0 0 0
P. laterouterus	0 9 0 0 1 0 1 0 0 0 1 9 0 0 0 0 0 1 1 2 0 2 0 1 0 0 1 0 0 1 1 0 0 2 0 0 0 0 0 2 1 2 0 1 0 0 0 0 0 0
P. linearis	9 1 0 1 1 0 1 1 3 0 1 9 9 9 0 0 0 1 1 0 1 0 1 0 1 0 0 0 0 1 1 0 1 0 0 0 0 0 0 2 1 2 0 1 0 0 0 0 0 0
P. malaclemys	0 2 0 0 1 0 1 1 3 0 1 1 9 0 0 0 0 1 1 0 0 2 0 0 1 0 0 1 0 1 9 1 0 2 0 1 9 0 0 2 1 2 0 1 0 0 0 0 0 0
P. mandapamensis	0 1 1 1 1 0 1 0 0 0 1 9 0 9 0 0 0 1 1 2 0 0 1 1 0 0 0 0 0 1 0 0 1 0 0 9 0 0 0 2 1 9 0 1 0 0 0 0 0 0
Notocotoyloides petasatum	1 3 1 2 1 0 1 0 0 0 1 0 0 3 0 0 0 0 2 2 0 0 0 0 0 0 1 0 9 9 9 9 1 1 0 1 9 0 0 2 1 3 0 1 0 0 0 0 0 0
Pyelosomum longicaecum	1 1 1 1 1 0 1 0 1 0 1 9 9 9 9 9 0 1 1 3 0 2 0 9 1 0 9 1 9 9 9 9 0 2 9 9 1 9 1 2 1 9 0 1 0 0 0 0 0 0
P. cochlear	1 2 0 1 1 0 1 0 1 0 1 1 0 3 0 0 0 1 1 1 0 0 1 0 2 0 0 9 0 1 0 1 0 2 0 0 1 0 1 2 1 2 0 1 0 0 0 0 0 0
P. parvum	1 3 1 2 1 1 1 0 1 0 1 9 9 9 0 0 0 1 1 2 9 1 0 0 2 0 1 2 0 1 0 1 1 2 0 9 0 0 1 2 1 9 0 1 0 0 0 0 0 0
Pyelosomum posterorchis	1 2 0 1 1 0 1 0 1 0 1 0 9 9 0 0 0 1 1 2 0 0 9 0 2 0 1 2 0 1 0 1 9 2 0 9 1 0 1 2 1 1 0 1 0 0 0 0 0 0
P. solum	1 9 9 1 1 0 1 0 1 0 1 9 9 3 0 9 0 0 9 1 9 2 1 9 9 9 1 2 0 1 0 1 9 2 0 0 0 0 1 2 1 1 0 1 0 0 0 0 0 0
P. renicapite	1 2 1 1 1 0 1 0 0 0 9 9 9 9 9 0 0 1 9 1 1 2 0 9 9 9 9 9 9 9 9 0 1 0 2 0 1 1 0 1 2 1 9 0 1 0 0 0 0 0
Myosaccus amblyrhynchi	1 3 1 1 1 1 1 0 1 0 1 0 0 3 0 0 0 1 1 2 0 1 0 1 0 0 0 0 0 1 1 0 0 1 0 9 9 0 0 2 1 1 0 1 0 0 0 0 0 0
M. chelonei	9 2 0 1 1 1 1 0 1 0 1 9 9 0 0 0 0 1 1 1 0 0 1 0 2 0 0 2 0 1 0 1 9 2 0 0 0 0 1 2 1 1 0 1 0 0 0 0 0 0
Cricocephalus albus	1 2 1 1 1 1 1 1 0 3 0 1 0 9 9 0 0 0 1 1 0 0 1 0 1 0 0 0 0 0 0 0 1 0 1 9 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0

TAXA	CHARACTERS 1	10	20	30	40	50
C. indicus	1 9 0 2 1 1 1 0 3 0 1 9 0 9 0 0 0 1 1 0 0 2 0 1 0 0 0 0 0 9 0 0 9 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
C. megastomus	1 2 1 2 1 1 1 0 2 0 1 0 0 3 0 0 0 1 1 2 0 1 0 1 1 0 0 1 0 0 1 0 1 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
C. resectus	1 2 1 2 1 1 1 1 3 0 1 0 9 9 9 0 0 1 1 0 0 2 0 1 0 0 0 0 0 0 0 1 0 1 4 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
C. koidzumii	1 2 1 1 1 1 1 1 0 3 0 1 9 9 9 0 0 0 1 9 0 9 2 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Pleurogonius minutissimus	0 2 0 1 1 0 1 1 3 0 1 0 9 9 9 9 0 1 1 0 0 0 9 1 1 0 1 1 1 0 1 9 1 1 0 0 9 2 0 0 2 1 1 0 1 0 0 0 0 0 0
Teloporia aspidonectes	1 3 1 2 1 0 1 0 1 0 2 1 0 3 0 0 2 0 1 1 0 1 0 1 0 0 0 0 0 1 0 0 1 1 0 0 0 0 0 2 1 2 0 1 1 0 0 0 0 0
Pronocephalus obliquus	1 1 0 0 1 0 1 0 0 0 2 1 0 3 0 0 1 1 1 2 0 0 1 1 0 1 0 0 0 1 1 0 1 0 0 0 1 0 0 2 1 1 0 1 1 0 0 0 0 0
Pronocephalus mehrai	9 1 0 0 1 0 1 0 0 0 2 9 0 0 0 0 1 1 1 2 0 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 2 1 1 0 1 1 0 0 0 0 0
Charaxicephalus robustus	1 2 1 1 1 3 1 0 2 0 2 0 9 9 0 1 0 0 1 1 0 0 0 0 1 0 9 1 0 1 9 1 1 0 0 0 0 0 0 2 1 9 0 3 0 0 9 9 1 0
C. loossi	1 2 1 1 1 3 1 0 2 0 2 1 9 9 0 0 0 1 1 2 0 0 0 1 0 0 2 0 0 1 0 1 1 0 0 9 1 0 0 2 1 1 0 3 0 0 9 9 0 0
Charaxicephaloides polyorchis	1 2 1 1 1 3 1 0 3 0 1 9 9 9 0 0 1 1 9 2 9 0 0 9 9 9 9 2 0 1 9 1 0 2 0 0 9 0 0 2 1 9 0 3 0 0 9 9 1 0
Desmogonius loossi	0 0 1 0 1 1 1 0 4 0 1 0 9 9 0 0 0 0 9 1 3 9 0 0 0 0 2 1 2 2 0 1 1 1 0 2 0 0 0 0 0 2 1 1 0 3 0 0 9 9 1 0
Diaschistorchis pandus	0 0 1 1 1 0 1 0 3 0 1 1 1 1 0 0 0 1 1 3 0 0 0 0 2 0 1 1 0 1 0 1 0 2 0 9 1 0 0 2 1 1 0 3 0 0 1 0 0 0
D. ellipticus	0 0 1 1 1 0 1 0 3 0 1 9 0 3 0 0 0 1 1 1 0 0 0 0 2 0 1 2 0 1 0 1 0 2 0 1 1 0 0 2 1 1 0 3 0 0 1 0 0 0
D. gastricus	1 3 1 1 1 1 1 1 0 1 0 1 0 9 9 0 0 0 1 1 1 0 0 0 0 1 0 2 2 0 1 9 1 9 0 0 0 1 0 0 2 1 1 0 3 0 0 1 1 0 0
D. kachugai	0 0 1 1 1 1 1 1 0 0 0 1 0 9 9 0 0 0 1 1 1 0 0 0 1 1 0 0 1 0 1 0 1 0 1 9 0 0 1 1 0 0 2 1 1 0 3 0 0 1 1 0 0
D. prafullai	0 0 1 0 1 1 1 0 3 0 1 9 9 9 0 0 0 0 0 1 3 0 0 0 0 2 0 0 2 0 1 0 1 0 2 0 0 1 0 0 2 1 1 0 3 0 0 2 9 0 0
D. singhi	1 3 1 1 1 0 1 0 1 0 1 9 1 9 0 0 0 1 1 3 0 0 0 0 9 0 2 1 0 1 0 1 9 2 0 1 1 0 0 2 1 1 0 3 0 0 1 1 0 0
D. lateralis	0 0 0 0 1 9 1 0 3 0 1 0 9 9 0 0 0 0 1 1 2 0 1 0 0 2 0 1 2 0 1 0 1 1 2 0 9 1 0 0 2 1 9 0 3 0 0 2 9 0 0
D. takahashii	1 3 1 1 1 1 1 1 0 1 0 1 0 9 9 0 0 0 0 0 1 1 0 0 0 0 1 0 2 1 0 1 0 1 0 2 0 9 1 0 0 2 1 1 0 3 0 0 1 1 0 0
Desmogonius desmogonius	0 0 0 0 1 1 1 0 3 0 1 0 9 9 0 0 0 0 9 1 2 0 0 0 0 2 0 2 2 0 1 1 1 1 2 0 0 0 0 0 2 1 1 0 3 0 0 9 9 1 0
Epibathra crassa	1 2 0 1 1 0 1 0 1 0 1 1 1 9 0 0 0 0 1 1 2 0 0 0 0 1 0 0 1 0 1 0 1 0 1 1 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Glyphicephalus lobatus	1 1 0 0 1 0 1 0 1 0 1 0 9 9 0 0 0 0 1 1 2 0 1 0 1 0 0 0 0 0 0 1 0 1 0 2 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Pleurogonius chelonei	1 1 1 0 1 0 1 0 1 0 1 9 9 3 0 0 0 0 1 1 1 0 0 0 0 1 0 9 0 0 0 0 1 9 0 9 1 0 9 0 0 0 2 1 1 0 1 0 0 0 0 0 0
Iguanacola navicularis	1 1 1 1 1 0 1 0 1 0 1 0 1 1 0 0 0 0 0 1 2 0 2 1 0 1 0 0 0 0 0 1 1 0 0 0 0 0 0 1 9 0 2 1 1 0 1 0 0 0 0 0 0
Pleurogonius carettae	1 1 0 0 1 0 1 0 0 0 1 9 0 0 0 0 1 0 1 1 1 0 0 0 1 0 9 0 0 0 0 1 9 0 9 1 0 9 0 0 0 2 1 1 0 1 1 0 0 0 0 0

TAXA	CHARACTERS 1 10 20 30 40 50
P. keamarii	1 1 0 0 1 0 1 0 1 0 1 9 0 9 0 1 0 1 1 1 0 0 0 0 0 0 0 0 0 0 1 9 0 1 1 0 0 0 0 0 2 1 1 0 1 1 0 0 0 0 0
Glyphicephalus solidus	1 1 1 1 1 0 1 0 3 0 1 0 9 9 0 0 0 1 1 2 0 0 0 1 0 0 0 0 0 0 1 9 0 0 0 0 9 0 0 0 2 1 1 0 1 1 0 0 0 0 0
Medioporus macrophallus	1 2 0 0 1 0 1 0 0 0 1 0 9 9 0 0 0 1 1 2 0 1 0 1 0 0 0 0 0 1 1 0 0 1 0 9 0 0 0 2 1 9 0 1 1 0 0 0 0 0
M. cheloniae	1 2 0 0 1 0 1 0 1 0 1 9 9 9 0 0 0 1 1 2 0 0 0 1 0 0 0 0 0 1 1 0 9 1 0 9 0 0 0 2 1 1 0 1 1 0 0 0 0 0
Pleurogonius americanus	1 2 0 0 1 0 1 0 0 0 9 0 0 9 0 0 0 1 1 2 0 0 0 0 1 0 2 1 0 1 1 0 9 1 9 9 1 0 0 2 1 1 0 1 0 0 0 0 0 0

Finally, a composite character representation of the genus *Rhabdiopoeius* Johnston, 1913, was included in the data set as an *outgroup taxon.* The outgroup taxon is a putative sister group, the morphology of which is used to determine the primitive or *plesiomorphic* character states of the taxa in question (= *ingroup taxa).* Any character state that occurs in both the outgroup and ingroup is hypothesised to be a plesiomorphic state. The determination of character polarity allows the tree to be *rooted*, so that cladogenetic events are represented in a temporal order.

The analysis is based on a total of 127 character states, of which 77 are derived states. Approximately ten percent of the character states of individual taxa were unknown or missing (i.e., coded "9"). For a tree with 89 terminal taxa to be fully resolved, and have no homoplasies, there must be at least as many derived characters as there are internal nodes on the tree. In this analysis, there would have to be at least 87 derived character states which are shared by at least two taxa. Therefore, insofar as the number of characters is concerned, the character-taxon data amassed in this study are potentially sufficient for a phylogenetic tree to be constructed with only a few unresolved branches.

Phylogenetic analysis

The phylogenetic reconstruction technique used here is an iterative weighted parsimony analysis, based on the technique developed by Farris (1969). The analysis involves the following steps:

1. An initial parsimony analysis is conducted.
2. The *unit character consistency indices*, c_i, are calculated by taking the ratio of the number of derived character states for each character and the number of times that character is hypothesised to have changed.
3. The weight for the *i*th character, w_i is calculated as

$$w_i = \{[\ (2m - 3)c_i\ ]^3 - 1\}/100,$$

where m is the number of taxa.

4. The weights are incorporated into another parsimony analysis. If the resulting tree is topologically identical to that of the previous run, the analysis is halted. Otherwise, Steps 2, 3, and 4 are repeated.

The parsimony analysis was carried out on a Digital Equipment MicroVAX II mainframe computer at the University of Canterbury, New Zealand, using PAUP (Phylogenetic Analysis Using Parsimony Ver 2.4.0 ; Swofford, 1984). The following options in PAUP were used:

ROOT=OUTGROUP, using *Rhabdiopoeius* as the outgroup to root the tree;
SWAP=GLOBAL for global branch swapping; and
CHGLIST to obtain the consistency index for each character.
I noted that after seven iterations of the analysis, the weights of 28 characters remained constant, but those of 22 characters fluctuated. Correspondingly, in the regions of the phylogenetic tree in which these 22 characters determined taxon groupings, the topology of the tree was unstable. Typically, these "unstable" characters had consistencies of 0.25 or less, i.e., for every derived state, at least four events of parallel evolution are recorded. Also, the number of characters that retained the same weight as they had in the run before, did not show an increasing trend (Fig. 6.10) as would be expected if the analysis was "converging" to some final tree. This suggested that the "unstable" characters could not be placed on a phylogenetic tree in an unambiguous and unequivocal pattern. The most likely reason for this is the number of unknown or missing character states.

As a measure of expediency, the analysis was modified so that the average of the weights of all characters were calculated on the basis of the weights of Runs 3 to 7. Obviously, this did not affect characters that had constant weights throughout these runs. The final weights are given in Column 6 of Table 6.3. A final analysis was conducted using these weights.

RESULTS AND DISCUSSION

The resultant phylogenetic tree is given in Fig 6.11, and the statistics of the tree together with those of the characters are given in Table 6.3. All but two of the characters that were weighted highly in the analysis (i.e., arbitrarily, weights greater than or equal to 197.9) in the analysis were considered to be well-defined. Because they were are not as susceptible to coding errors and errors of interpretation as questionable characters, I place a high confidence value on the monophyly of those groups defined by these characters.

While many pronocephalid genera erected and described by taxonomists are indeed monophyletic, others, notably genus-members of the sub-family Pronocephalinae Looss, 1899 are para- or polyphyletic. They include the genera *Pleurogonius*, *Epibathra*, *Barisomum*,*Glyphicephalus*, *Myosaccus* Gilbert, 1936. As early as 1932, R.K. Mehra recommended the amalgamation of *Pleurogonius* and *Glyphicephalus*, and in 1981, H.R. Mehra synonymised *Pleurogonius*, *Epibathra*, *Barisomum*, *Glyphicephalus* and *Medioporus* Oguro, 1936. In the next chapter, the applicability of these classifications will be discussed in the light of the phylogenetic hypothesis presented here.

Figure 6.10 The number of characters which remain unstable after each run of PAUP. After the third run, this number remains relatively constant.

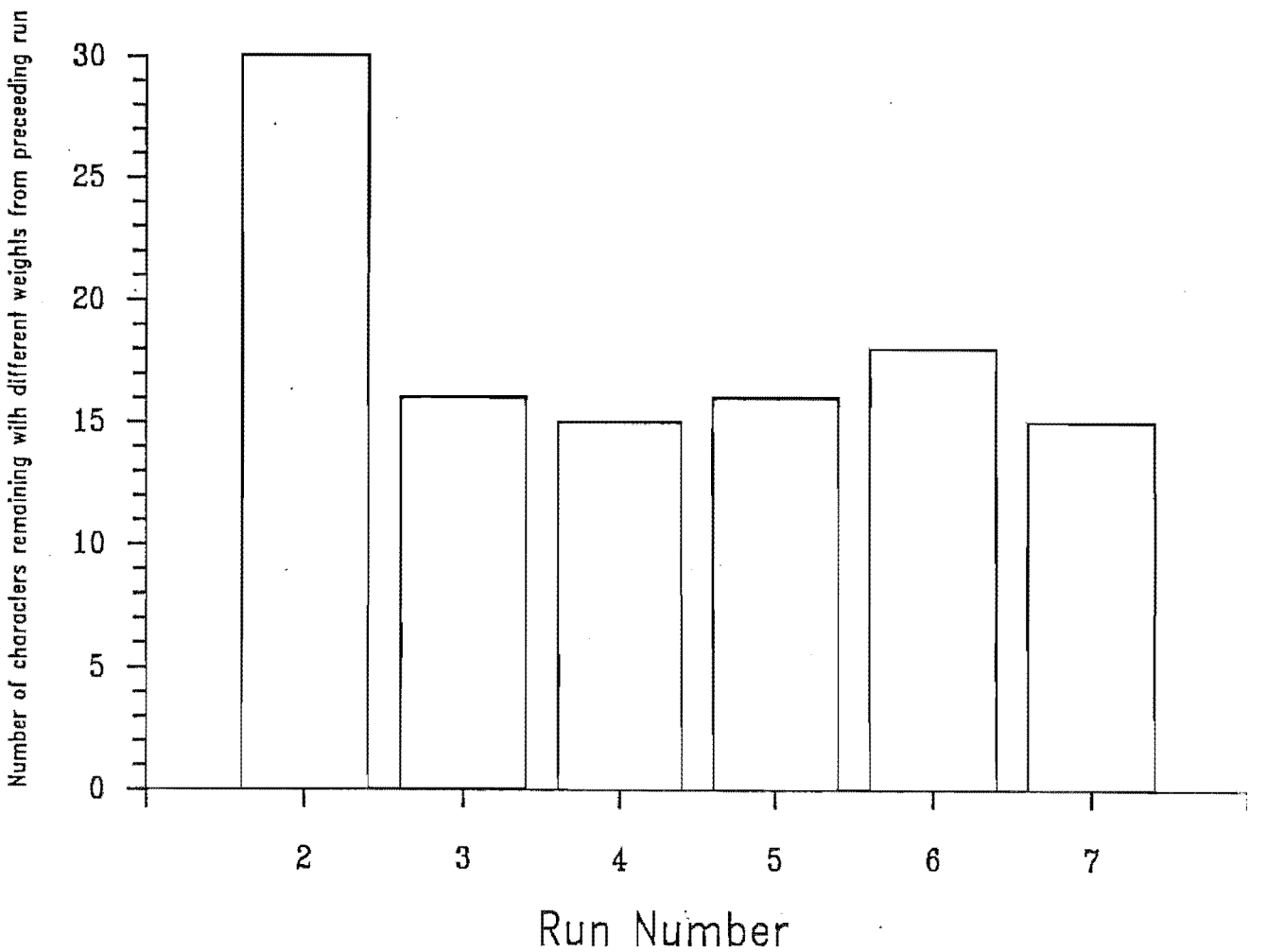
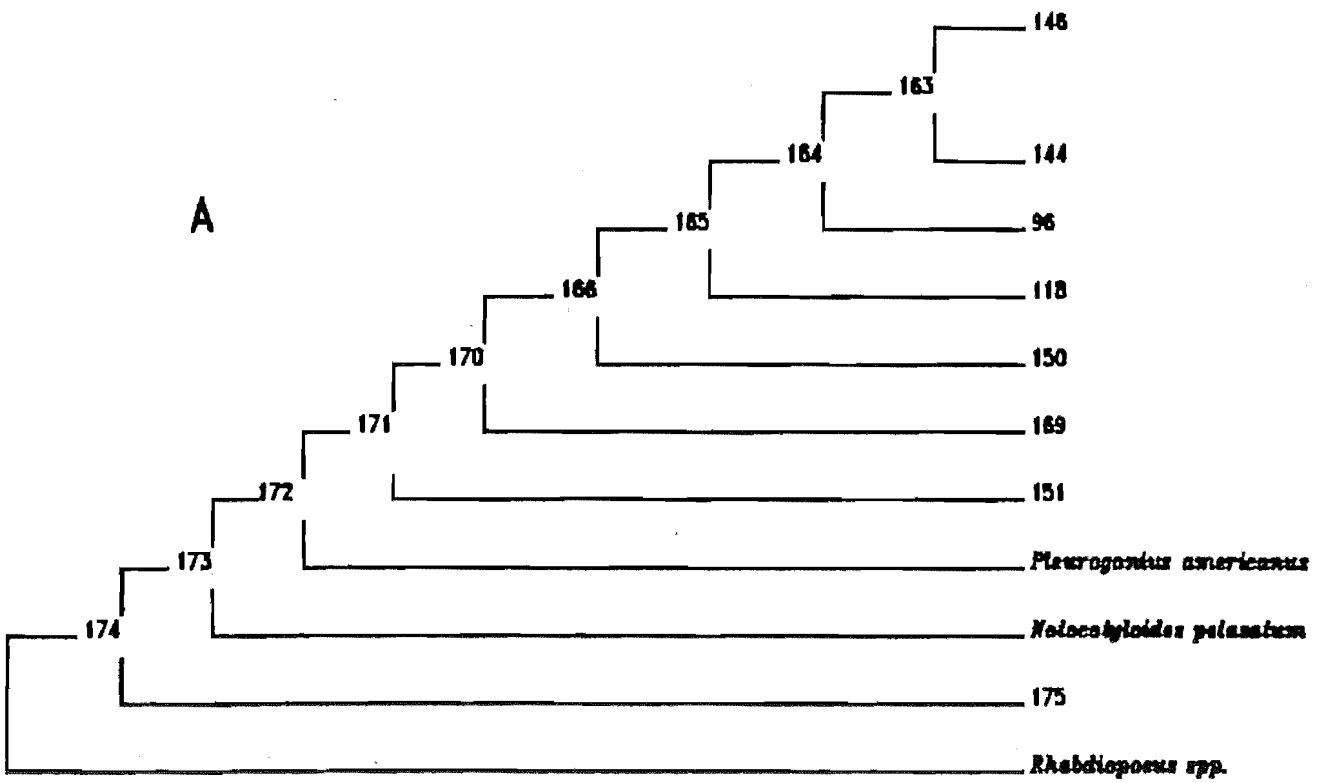
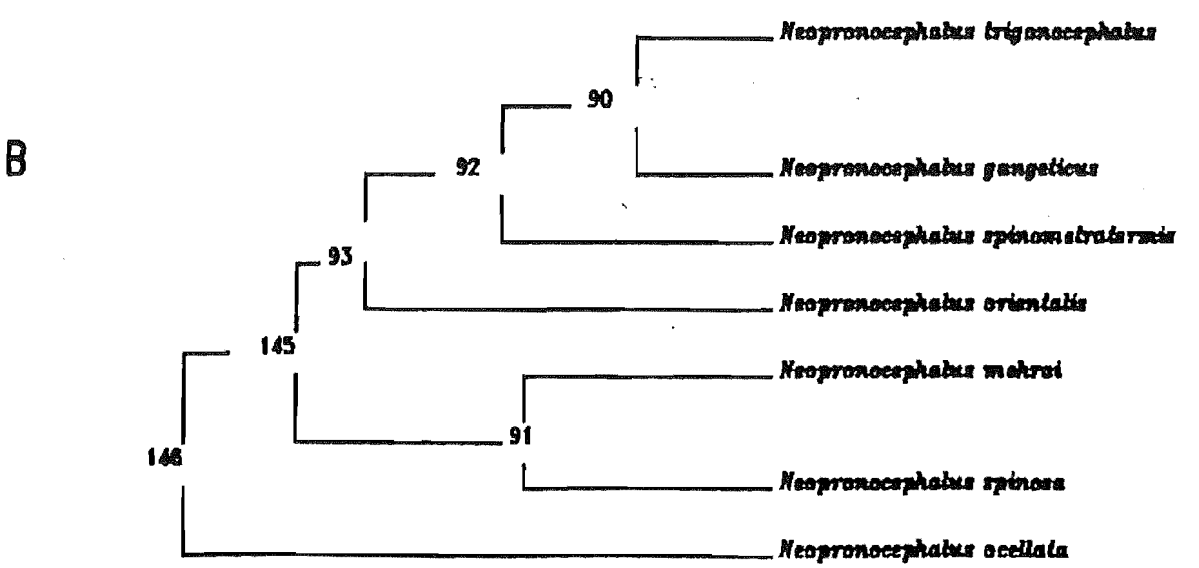


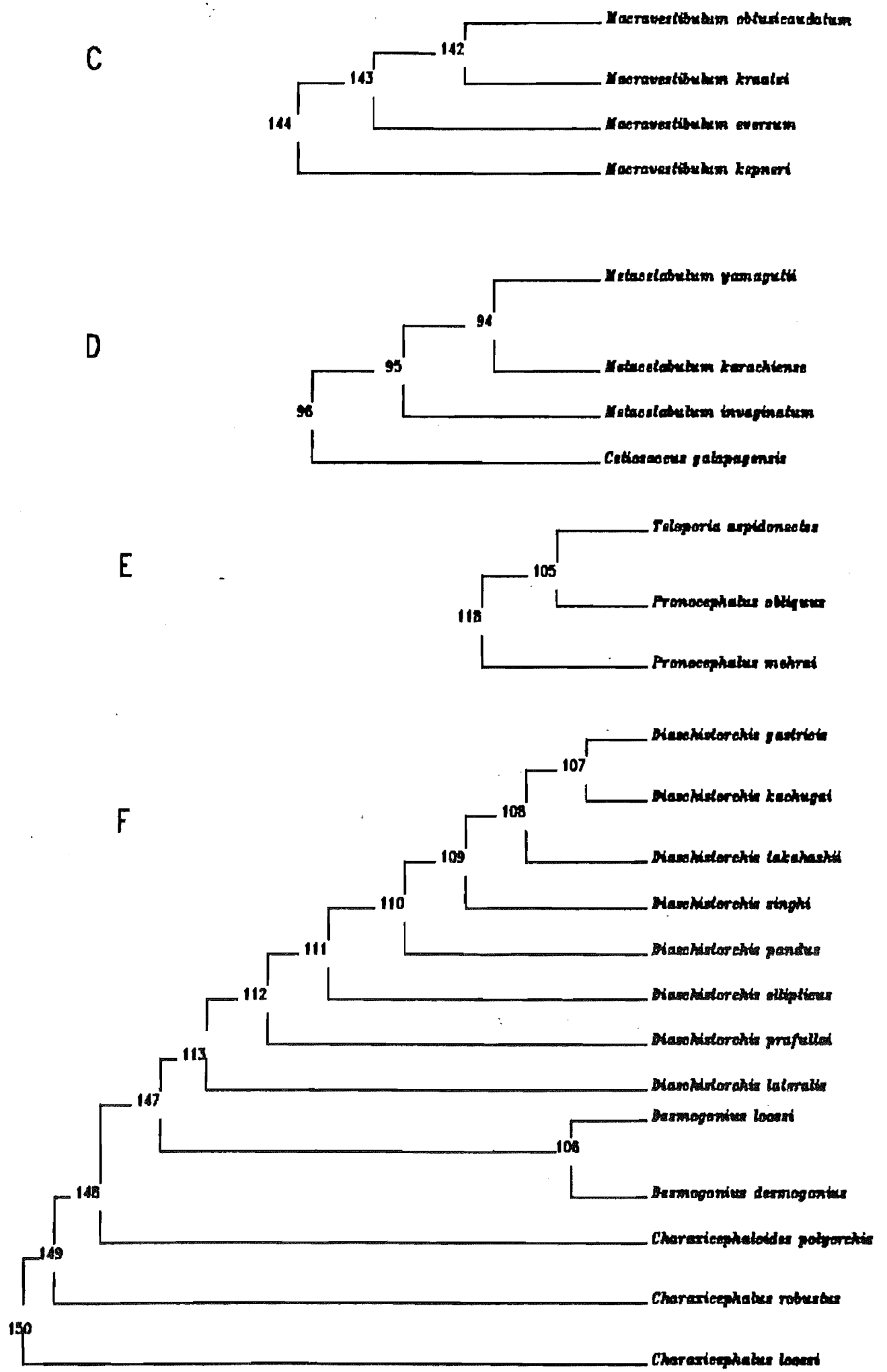
Table 6.3 Statistics relating to the characters used in the phylogenetic analysis of the Pronocephalidae. These include the ancestral state of each character (equivalent to the state of the outgroup) and the final weight of each character.

Character	Number of states	Ancestral state	Number of changes	Consistency Index	Final Weight	Status
1	2	0	15	0.067	2.31	Q
2	5	0	20	0.2	43.58	Q
3	2	0	26	0.038	0.38	Q
4	3	0	21	0.095	4.21	Q
5	2	0	1	1.0	5359.37	W
6	3	0	9	0.222	49.18	Q
7	2	1	2	0.5	669.92	W
8	2	0	5	0.2	42.87	Q
9	4	0	22	0.182	32.38	W
10	3	0	2	1.0	5359.37	W
11	3	0	4	0.5	669.92	W
12	2	0	7	0.143	11.92	Q
13	2	0	3	0.333	197.9	Q
14	4	0	11	0.273	116.7	Q
15	2	0	1	1.0	5359.37	Q
16	2	0	6	0.167	23.1	W
17	3	0	5	0.4	343.0	W
18	2	1	12	0.083	3.06	W
19	2	2	1	1.0	5359.37	W
20	4	0	25	0.12	7.5	W
21	2	0	8	0.125	10.47	W
22	3	2	27	0.074	2.28	Q
23	2	1	12	0.083	2.69	W
24	2	0	17	0.059	1.14	Q
25	3	0	16	0.125	12.33	Q
26	2	1	9	0.111	7.56	Q
27	3	1	23	0.087	3.73	W
28	3	0	20	0.1	5.52	Q
29	2	1	5	0.2	42.87	W
30	2	1	2	0.5	669.92	W
31	2	0	17	0.059	1.1	W
32	2	0	15	0.067	1.56	Q
33	2	1	16	0.063	1.39	Q
34	4	4	29	0.103	4.99	W
35	3	0	2	1.0	5359.37	Q
36	2	0	13	0.077	2.45	Q
37	3	1	15	0.133	14.45	Q
38	2	0	2	0.5	669.92	W
39	2	0	1	1.0	5359.37	W
40	3	2	3	0.667	1590.35	W
41	3	1	2	1.0	5359.37	W
42	4	3	8	0.375	282.62	W
43	2	0	1	1.0	5359.37	W
44	2	1	1	1.0	5359.37	W
45	2	0	5	0.2	35.71	W
46	2	0	1	1.0	5359.37	W
47	3	0	2	1.0	5359.37	W
48	2	0	1	1.0	5359.37	W
49	2	0	2	0.5	669.92	W
50	2	0	1	1.0	5359.37	W

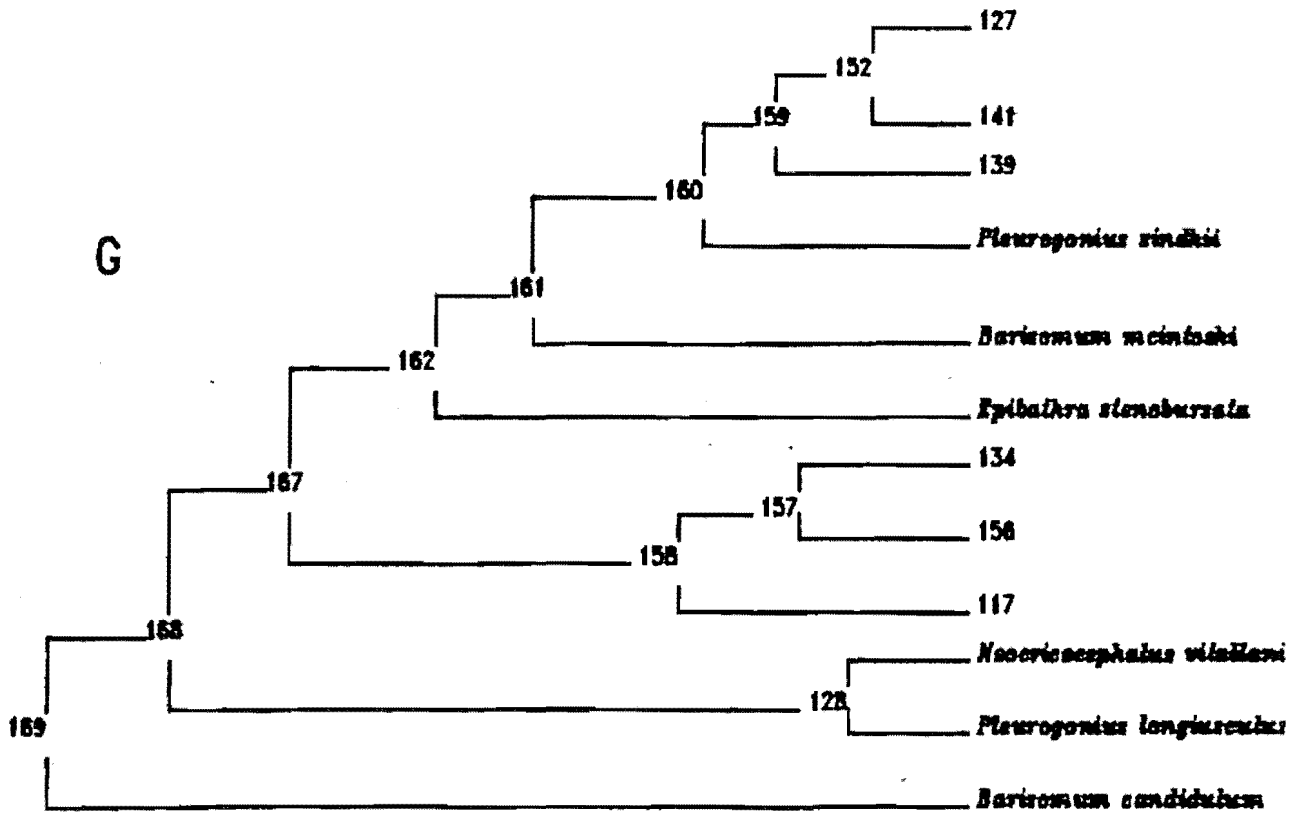
Figure 6.11 The phylogeny of the Pronocephalidae. Because of the number of taxa, the phylogenetic tree has been broken up into clades. Numbers represent the nodes of the phylogenetic tree and indicate where each clade joins the tree. Character changes along each branch are listed in Table 6.4.



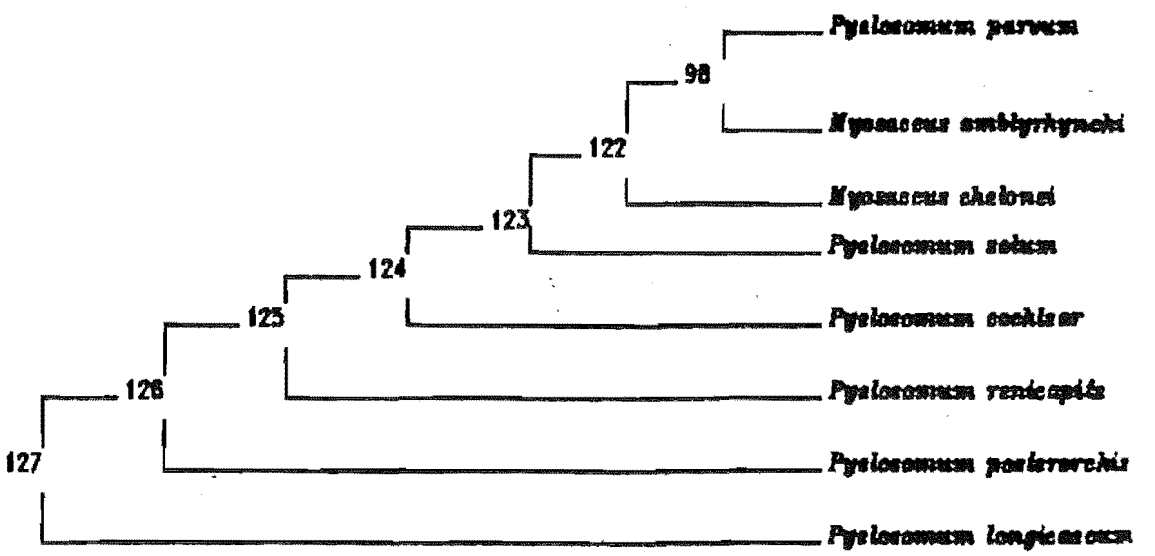



C
142
143
144
Macravestibulum obtusicaudatum
Macravestibulum krusei
Macravestibulum everum
Macravestibulum kapseri
D
94
95
96
Metacetabulum yamaguti
Metacetabulum karachiense
Metacetabulum invaginatum
Catiosaccus galapagensis
E
105
118
Telogaria aspidonectes
Pronocephalus obliquus
Pronocephalus mehrai
F
107
108
109
110
111
112
113
147
148
149
150
106
Diaschistorchis gastricis
Diaschistorchis kachugai
Diaschistorchis takahashii
Diaschistorchis singhi
Diaschistorchis pandus
Diaschistorchis ellipticus
Diaschistorchis prafullai
Diaschistorchis latiralis
Barmogonius loossi
Barmogonius dermogonius
Charaxicephaloides polyorchis
Charaxicephalus robustus
Charaxicephalus looss

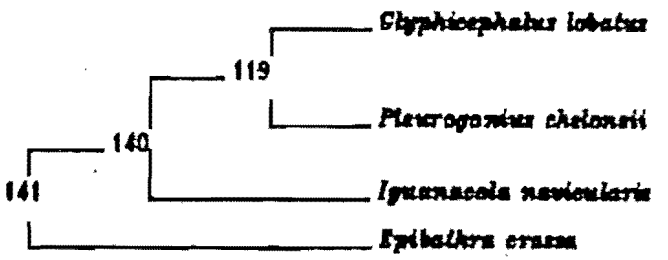
G


127
152
159
141
139
160
Pleuroganius sinalkii
161
Bariconum mcintoshi
162
Epibalbra stenoburnia
167
134
157
156
158
117
168
Neocricocephalus williami
128
Pleuroganius longisuturalis
169
Bariconum candidulum

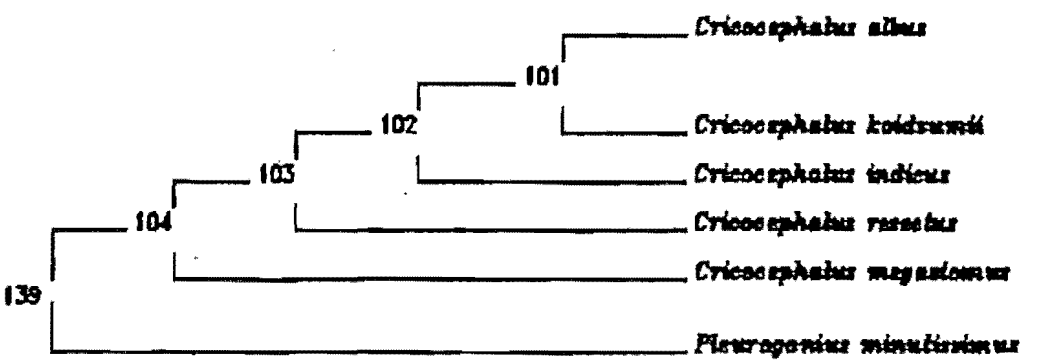
G(a)


Pyloconcum parvum
98
Mycoaceus amblyrhynchi
122
Mycoaceus chelonei
123
Pyloconcum solum
124
Pyloconcum cochlear
125
Pyloconcum venicapita
126
Pyloconcum pachyrrachis
127
Pyloconcum longicaecum

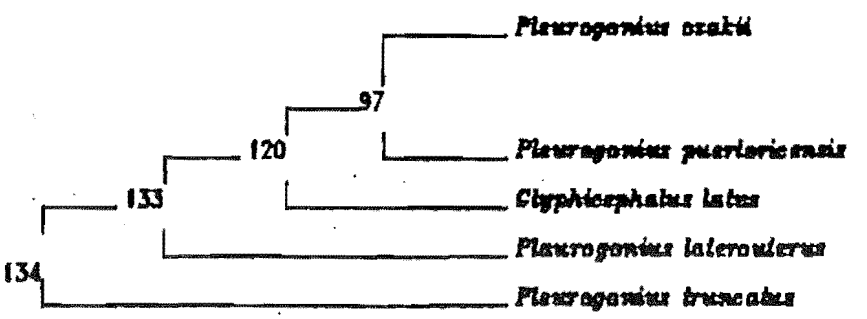
G(b)


Glyphicephalus lobatus
119
Pleurogonius chelonii
140
Iguanacola navicularis
141
Epibathra crassa

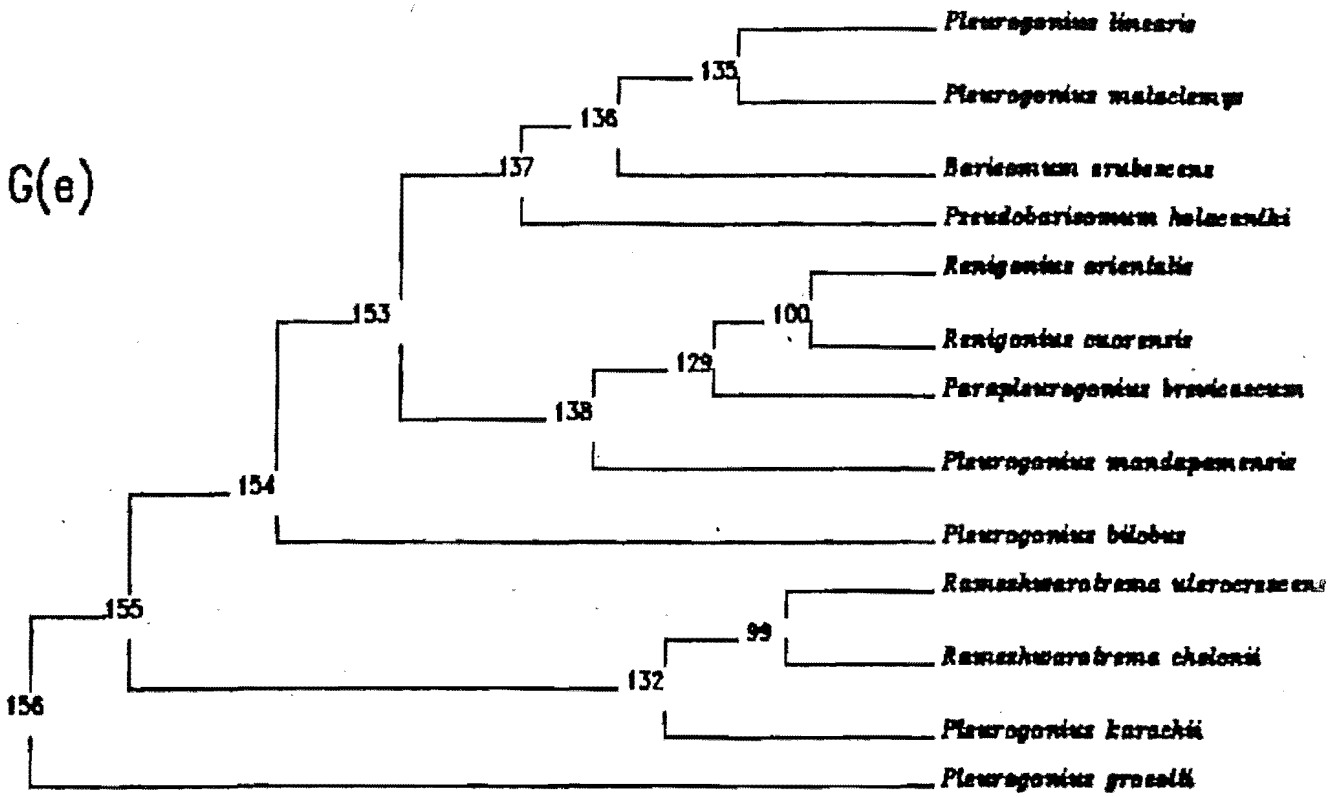
G(c)


Cricocephalus albus
101
Cricocephalus koidzumii
102
Cricocephalus indicus
103
Cricocephalus rusatus
104
Cricocephalus megastomus
139
Pleurogonius minutissimus

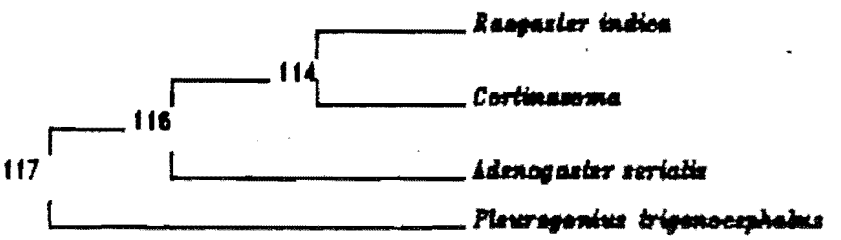
G(d)


Pleurogonius ozakii
97
Pleurogonius puerlaricensis
120
Glyphicephalus latus
133
Pleurogonius laterouterus
134
Pleurogonius truncatus

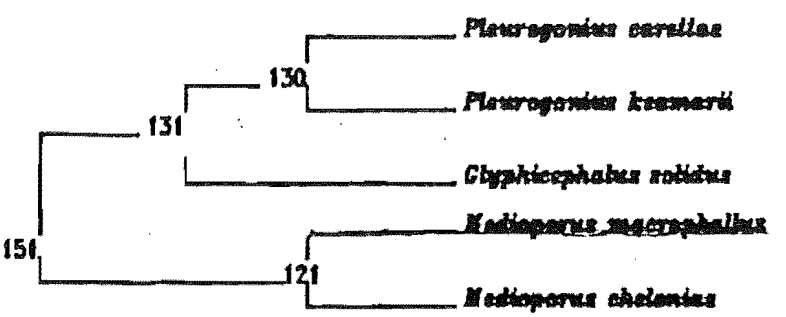
G(e)


Pleurogenius linearis
135
Pleurogenius malaclemys
136
Bariosomum erubescens
137
Pseudobariosomum holacanthi
Renigonius orientalis
153
100
Renigonius ouroensis
129
Paraplesiogonius brevicaecum
138
Pleurogenius mandapamensis
154
Pleurogenius bilobus
Ramashwarabrema ulerocrucens
155
99
Ramashwarabrema chalonii
132
156
Pleurogenius karachii
Pleurogenius grossii

G(f)


Rangazier indica
114
Cortinasoma
116
117
Adenogaster serialis
Pleurogenius trigonocephalus

H


Pleurogenius carolinae
130
Pleurogenius kesmarii
131
Glyphicephalus solidus
Medioporus macrophallus
156
121
Medioporus chelonius

I

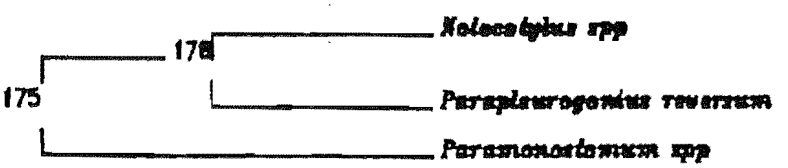

Nolacalpus spp
178
175
Paraplesiogonius texarum
Paramonostomum spp

Table 6.4 List of character changes along each branch of the pronocephalid phylogenetic tree. The numbers in column 1 refer to the nodes of the tree given in Figure 6.11.

Nodes	Characters	Changes
174 - *Rhabdiopoeius*	11	1->5
	20	2->0
	22	0->2
	23	0->1
	29	0->1
	31	1->0
	34	1->4
175 - *Paramonostomum*	24	0->1
90 - *Neopronocephalus triangularis*	*no changes*	
90 - *N. gangeticus*	*no changes*	
91 - *N. mehrai*	8	0->1
	25	0->2
	34	2->0
146 - *N. ocellata*	8	0->1
	9	0->1
92 - *N. orientalis*	30	1->0
	31	1->0
	35	0->2
91 - *N. spinosa*	24	1->0
93 - *N. wamani*	*no changes*	
143 - *Macravestibulum eversum*	36	0->1
144 - *M. kepneri*	*no changes*	
96 - *Cetiosaccus galapagensis*	38	0->1
	33	0->1
	30	1->0
	29	0->1
	27	0->1
	23	0->1
	9	0->1
	3	0->1

...cont'd

Node	Character	Changes
128 - *Neocricocephalus vitallani*	4	0->2
	16	0->1
	20	2->0
	22	0->2
	23	0->1
	36	0->1
128 - *Pleurogonius longiusculus*	1	1->0
	36	2->0
	37	0->1
172 - *P. americanus*	25	0->1
	28	0->1
154 - *P. bilobus*	26	0->1
	37	0->1
130 - *P. carettae*	*no changes*	
119 - *P. chelonei*	20	2->1
	34	2->1
156 - *P. grocotti*	18	1->0
	26	0->1
	34	0->1
	37	0->1
132 - *P. karachii*	27	1->2
	28	0->1
130 - *P. keamarii*	9	0->1
	24	1->0
133 - *P. laterouterus*	22	1->2
	34	0->2
135 - *P. linearis*	1	0->1
	21	0->1
	22	2->0
	23	0->1
	28	1->0
	32	1->0
	34	2->0
135 - *P. malaclemys*	2	1->2
	4	1->0
	33	1->0
	36	0->1

...cont'd

Node	Character	Changes
95 - *Metacetabulum invaginatum*	18	0->1
	21	0->1
	24	1->0
	25	0->2
	26	0->1
	35	0->1
	37	1->0
94 - *M. yamagutii*	*no changes*	
94 - *M. karachiense*	33	0->1
	34	2->0
176 - *Parapleurogonius brevicaecum*	*2*	*0->4*
	4	*0->2*
	9	0->3
	22	0->1
127 - *Pyelosomum longicaecum*	2	2->1
	22	0->2
112 - *Diaschistorchis prafullai*	18	1->0
	27	1->0
109 - *D. singhi*	*no changes*	
113 - *D. lateralis*	3	1->0
	22	0->1
	33	0->1
108 - *D. takahashii*	18	1->0
97 - *Pleurogonius ozakii*	*no changes*	
97 - *P. puertoricensis*	1	1->0
	21	0->1
	34	0->2
160 - *P. sindhi*	21	0->1
	27	0->2
	29	0->1
	34	2->0
	36	0->1
117 - *P. trigonocephalus*	27	1->0
	28	0->2
134 - *P. truncatus*	4	0->1
	6	0->1

...cont'd

Node	Character	Changes
137 - *Pseudobarisomum holacanthi*	*2*	*1->0*
	4	*1->0*
	22	2->1
	23	0->1
	45	0->1
124 - *Pyelosomum cochlear*	42	1->2
173 - *Notocotyloides petasatum*	3	0->1
	4	0->2
	14	0->3
	18	1->0
	36	0->1
98 - *Pyelosomum parvum*	4	1->2
	27	0->1
	33	0->1
126 - *P. posterorchis*	27	0->1
123 - *P. solum*	18	1->0
	27	0->1
99 - *Rameshwarotrema uterocrescens*	45	0->1
99 - *R. chelonei*	*no changes*	
100 - *Renigonius orientalis*	3	0->1
	4	1->0
	9	0->1
	31	0->1
100 - *R. cuorensis*	20	2->1
	28	0->1
101 - *Cricocephalus albus*	22	2->1
	31	0->1
102 - *C. indicus*	3	1->0
104 - *C. megastomus*	9	3->2
	20	0->2
	22	2->1
103 - *C. resectus*	8	0->1
	34	2->4
101 - *C. koidzumii*	*no changes*	

...cont'd

Node	Character	Changes
105 - *Teloporia aspidonectes*	2	1->3
	3	0->1
	4	0->2
	9	0->1
	18	1->0
	20	2->1
	22	0->1
	34	0->1
	42	1->2
149 - *Charaxicephalus robustus*	16	0->1
	18	1->0
	20	2->1
	25	2->1
	28	2->1
150 - *C. loossi*	37	0->1
148 - *Charaxicephaloides polyorchis*	17	0->1
106 - *Desmogonius loossi*	9	3->4
	20	2->3
	26	0->1
110 - *Diaschistorchis pandus*	*no changes*	
111 - *D. elliptica*	20	3->1
107 - *D. gastricus*	28	1->2
	36	1->0
107 - *D. kachugai*	1	1->0
	2	3->0
	9	1->0
	24	0->1
	27	2->0
116 - *Adenogaster serialis*	2	2->1
114 - *Raogaster indica*	36	0->1
	37	0->1
	42	2->1
105 - *Pronocephalus obliquus*	23	0->1
	26	0->1
	31	0->1
	37	0->1
118 - *P. mehrai*	27	0->1

...cont'd

Node	Character	Changes
125 - *Pyelosomum renicapite*	3	0->1
	9	1->0
	21	0->1
	22	0->2
	36	0->1
136 - *Barisomum erubescens*	3	0->1
	14	0->1
	25	1->2
	28	1->2
169 - *B. candidulum*	22	0->1
161 - *B. mcintoshi*	*no changes*	
141 - *Epibathra crassa*	3	1->0
	12	0->1
	33	0->1
162 - *E. stenobursata*	*no changes*	
131 - *Glyphicephalus solidus*	3	0->1
	4	0->1
	9	0->3
	34	1->0
119 - *G. lobatus*	3	1->0
	22	0->1
	32	0->1
120 - *G. latus*	3	0->1
	4	0->1
	14	0->3
121 - *Medioporus macrophallus*	22	0->1
121 - *M. cheloniae*	9	0->1
98 - *Myosaccus amblyrhynchi*	24	0->1
	25	2->0
	28	2->0
	31	0->1
	32	1->0
	34	2->1
122 - *M. chelonei*	14	3->0

...cont'd

Node	Character	Changes
138 - *Pleurogonius mandapamensis*	*3*	*0->1*
	23	*0->1*
	34	2->0
139 - *P. minutissimus*	1	1->0
	3	1->0
	8	0->1
	27	0->1
	34	2->0
	37	0->2
140 - *Iguanacola navicularis*	14	3->1
	18	1->0
	22	0->2
	23	0->1
	31	0->1
	34	2->0
	37	0->1
106 - *Desmogonius desmogonius*	3	1->0
	33	0->1
114 - *Cortinasoma ocadiae*	3	0->1
	4	0->1
	20	1->0
	21	0->1
	23	0->1
142 - *Macravestibulum obtusicaudatum*	*6*	*0->1*
142 - *M. kraatzi*	31	1->0
92 - 90	3	0->1
	27	2->0
145 - 91	3	0->1
93 - 92	6	0->1
145 - 93	16	1->0
	21	1->0
	27	0->2
	32	1->0
95 - 94	20	0->2
	22	0->2

...cont'd

Node	Character	Changes
96 - 95	1	1->0
	2	2->0
	14	2->1
	32	1->0
	34	0->2
	36	0->1
164 - 96	5	1->2
	14	0->2
	15	0->1
	17	1->2
	37	0->1
120 - 97	37	0->1
122 - 98	2	2->3
	3	0->1
	20	1->2
	22	0->1
	23	1->0
132 - 99	1	0->1
	2	0->3
	9	3->0
	16	0->1
	20	2->0
	22	0->2
	31	1->0
	34	0->1
129 - 100	2	1->3
	24	1->0
	26	0->1
	27	0->1
	29	0->1
	32	0->1
	34	2->4
102 - 101	4	2->1
	34	2->0
103 - 102	31	1->0
104 - 103	25	1->0
	28	1->0
139 - 104	4	1->2
	6	0->1
	22	0->2
	30	1->0
	32	1->0

...cont'd

Node	Character	Changes
140 - 119	4	1->0
	24	0->1
	25	1->0
133 - 120	1	0->1
	20	2->1
	27	1->0
151 - 121	*no changes*	
123 - 122	6	0->1
124 - 123	37	1->0
125 - 124	23	0->1
126 - 125	20	2->1
127 - 126	3	1->0
	25	1->2
	28	1->2
152 - 127	37	0->1
	39	0->1
168 - 128	2	2->3
	3	0->1
	25	0->1
138 - 129	1	0->1
	10	0->3
	33	1->0
131 - 130	16	0->1
	20	2->1
	33	0->1
151 - 131	2	2->1
155 - 132	29	0->1
	32	0->1
	36	0->1
134 - 133	9	1->0
	24	0->1
157 - 134	22	0->1
	33	1->0
136 - 135	8	0->1
	24	1->0

...cont'd

Node	Character	Changes
137 - 136	32	0->1
153 - 137	20	2->0
	22	0->2
	25	0->1
	28	0->1
153 - 138	9	3->0
	31	1->0
159 - 139	9	1->3
	20	2->0
	24	0->1
	33	0->1
141 - 130	2	2->1
	28	1->0
	32	1->0
152 - 141	13	0->1
143 - 142	*no changes*	
144 - 143	3	0->1
	28	1->0
	32	1->0
	33	0->1
163 - 144	4	0->1
	24	1->0
	38	0->1
146 - 145	46	0->1
163 - 146	11	2->1
	17	1->0
	18	0->1
	21	0->1
	22	0->2
	23	0->1
	34	0->2
	50	0->1
	45	1->0
148 - 147	1	1->0
	2	2->0
	4	1->0
	6	3->0

...cont'd

Node	Character	Changes
149 - 148	9	2->3
	11	2->1
	33	1->0
	34	0->2
150 - 149	12	1->0
	24	1->0
	25	0->2
	28	0->2
	49	0->1
166 - 150	3	0->1
	4	0->1
	6	0->3
	9	0->2
	14	0->3
	27	0->2
	44	1->3
	47	0->2
171 - 151	33	1->0
	45	0->1
159- 152	31	1->0
154 - 153	12	0->1
	27	1->0
	34	0->2
155 - 154	4	0->1
	24	0->1
156 - 155	9	1->3
157 - 156	2	2->1
158 - 157	24	1->0
	42	1->2
167 - 158	1	1->0
	34	2->0
160 - 159	4	0->1
161 - 160	3	0->1
	22	2->0
	25	2->1
	28	2->1
	32	0->1

...cont'd

Node	Character	Changes
162 - 161	24	1->0
	25	0->2
	27	1->0
	28	0->2
167 - 162	14	0->3
	22	0->2
	33	1->0
164 - 163	28	0->1
	41	3->2
	43	0->1
165 - 164	10	0->2
	16	0->1
	18	1->0
	20	2->0
	31	0->1
	33	1->0
	41	1->3
	42	1->0
166 - 165	17	0->1
	45	0->1
170 - 166	11	1->2
	31	1->0
	32	0->1
	34	2->0
168 - 167	12	1->0
169 - 168	27	0->1
170 - 169	9	0->1
171 - 170	12	0->1
	34	1->2
172 - 171	24	0->1
	27	2->0
	37	1->0
173 - 172	2	3->2
	19	2->1
	27	1->2
	42	3->1
174 - 173	1	0->1
175 - 174	3	1<-0
	13	1<-0
	18	1<-0
176 - 175	7	0<-1
	40	0<-2

Node	Character	Changes
Notocotylus spp. - 176	3	1->0
	31	1->0

...end

The analysis has also shed light on other interesting taxonomic problems. According to the phylogenetic hypothesis presented here, members of the Notocotylidae form a separate clade that emerges at the bottom of the tree. This suggests that the family forms a legitimate sister-group to the Pronocephalidae. Also, the position of *Parapronocephalum* Belpolskaia, 1952, has been resolved. It was suggested that the muscular cephalic modification of *Parapronocephalum* meant that the genus was a taxon intermediate between the notocotylids and the pronocephalids. However, Sinclair (1972) disputed this and demonstrated that the cephalic musculature was in fact not homologous to that of the pronocephalids. He placed *Parapronocephalum* in the Notocotylidae. The analysis presented here supports his view (the placement of *Parapronocephalum* is discussed in greater detail in Chapter 8).

Many taxonomists (e.g. Yamaguti, 1972) have also considered that the genera *Adenogaster* Looss, 1901, *Raogaster* (Lakshman Rao, 1975) Groschaft and Tenora, 1981 and *Cortinasoma* Oshmarin and Zharikova, 1984 all of which possess ventral papillae, are intermediate forms between the notocotylids and pronocephalids. Groschaft and Tenora (1981) even placed this group of genera and *Parapronocephalum* in a new family Parapronocephalidae. According to my analysis, however, ventral glands arose independently in the pronocephalids and notocotylids. This is not unlikely as some genera of the Paramphistomatata Skrjabin and Schulz, 1937, (*Gastrodiscus* and *Homalogaster*), a distome order, also possess dermal glands. It is possible that the multiple origins of these ventral glandular papillae is an indication of an *underlying synapomorphy* i.e., a synapomorphy which represents a predisposition to express a particular character state. Certainly, the different arrangements of the papillae in genera of the Notocotylidae suggest that the trait is subject to some structural plasticity (Beverly-Burton, 1972).

The hypothesis of multiple origins of ventral glands brings the following question to the fore: how can hypotheses of character evolution, which are the *auxilliary hypotheses* of a phylogenetic analysis, be tested ? I have discussed this, in general terms, in Chapters 2 and 4. In all instances, structural and ontogenetic evidence can lend support to, or lower our confidence in, character hypotheses of homology or analogy. Consider again the presence or absence of ventral glands. As stated above, the presence of this feature is often considered a good indicator of monophyly. However, the results of my analysis suggest that this trait arose in notocotylids and some pronocephalid genera. If the ultrastructure of the glands of these two

groups is similar, and if the immature adult stages of both notocotylids and pronocephalids (genera with and without glands) show the presence of these glands or gland precursors, there is sufficient evidence to cast doubt on the hypothesis of parallel evolution. In such an instance, the character states would have to be re-expressed to include the observation that ventral glands (or gland precursors) are present in immature stages and persist or are reduced at maturity. In other words, the data would have to be reanalysed taking these new character states into account. To date, no work has been done on the ultrastructure of the ventral glands of pronocephalids and what few life history studies have been undertaken on the pronocephalid reveal no evidence of ventral glands (Horsfall, 1930; Thapar, 1968; Saxena, 1977).

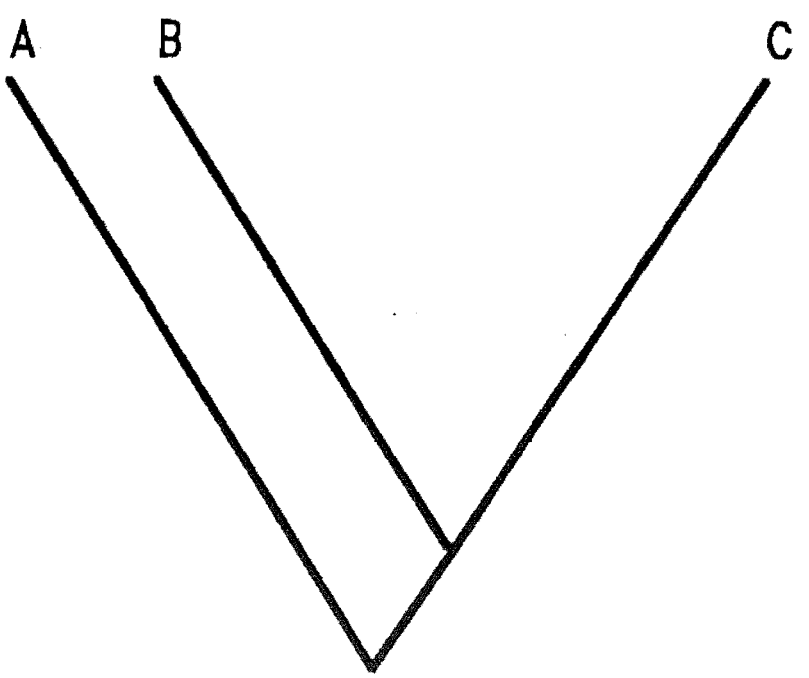
In much the same way, hypotheses of evolution of other characters can be scrutinised. In effect, these hypotheses form the basis of observational tests which would lead to a rejection of the phylogenetic hypothesis, at least with respect to the groups defined by these characters. Therefore, the hypotheses of character evolution identified here, can be used to direct research on the developmental biology and comparative morphology of the Pronocephalidae and related groups.

However, it was my main aim in reconstructing the phylogeny of the Pronocephalidae to identify a framework upon which the classification of the group can be developed. In the next chapter I discuss the protocol I used to translate the phylogenetic tree into a classification system. This system is presented in the final chapter (Chapter 8).

CHAPTER 7

TRANSLATING THE PHYLOGENY OF THE PRONOCEPHALIDAE INTO A CLASSIFICATION: TWO CRITERIA FOR GENERATING ACCEPTABLE PARAPHYLETIC CLASSIFICATIONS

Figure 7.1 Hypothetical phylogeny of three taxa, *A*, *B*, and *C*. The branch lengths represent the number of character changes. *A* and *B* are more similar to each other than to *C*. However, *A* and *B* constitute a paraphyletic group, while *B* and *C* are monophyletic.



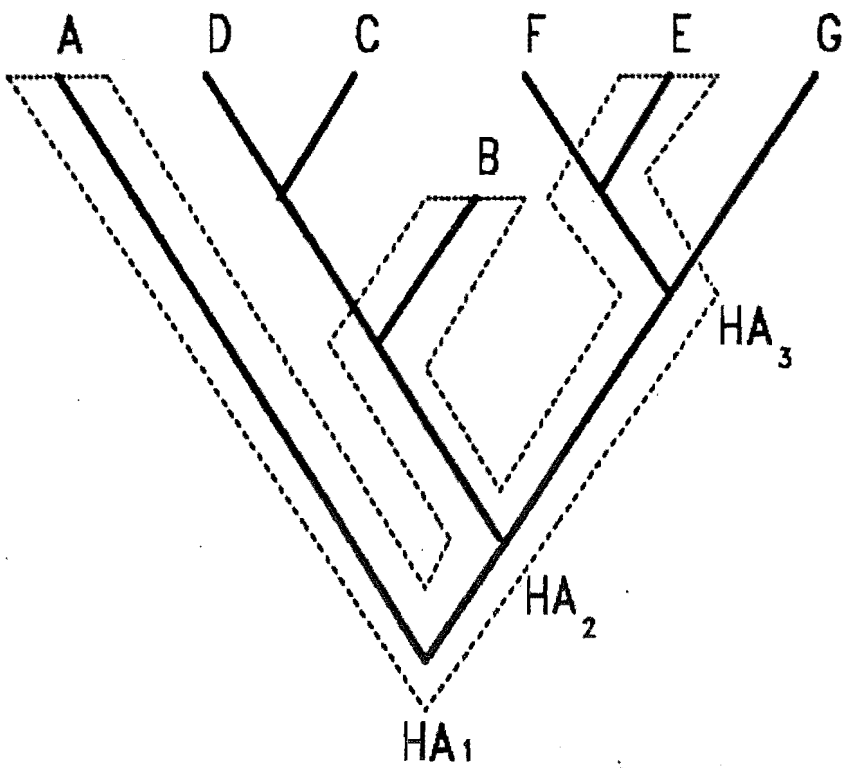
INTRODUCTION

Systematists who hold that taxonomic relationships should reflect phylogeny are nonetheless divided about what is the best way to translate a phylogenentic hypothesis into a classification. Since a phylogenetic hypothesis offers information on both anagenetic and cladogenetic events, there are potentially two types of information that can be maximised in a classification. In Fig. 7.1, A is closer to B than B is to C. However, B and C form a monophyletic group, whereas A and B are paraphyletic (I use the terms *monophyletic* and *paraphyletic* in the same sense as the cladists do). Classifying A and B results in a grouping which optimises taxonomic similarity. However, in doing so, the ability to "retrieve" the phylogenetic tree from the classification is forfeited. On the other hand, classifying B with C, or treating A, B, and C as separate taxa, allows the retrieval of *phylogenetic* information, but sacrifices taxonomic information.

The methods for translating a phylogenetic tree into a classification which preserves phylogenetic information were reviewed in Chapter 2. To construct a paraphyletic classification, Estabrook (1978) developed an interesting protocol based on the principle of "convexity" (Fig. 7.2). A classification is convex if the hypothetical ancestors of any taxa included in a larger polytypic super-taxon are not included in another super-taxon (Note: the term *super-taxon* refers to any taxon that is of a higher taxonomic rank than that of the terminal taxa of a phylogenetic tree). This restriction does not apply to monotypic super-taxa. Of course, hypothetical ancestors are never included in taxa, and this statement is a way of describing the process of transcribing the tree. In Fig. 7.2, A, B, and E make up a paraphyletic group. The group includes the hypothetical ancestors of A (= HA_1), B, C, and D (HA_2), and E and F (HA_3). If C, D, and F are included in a separate taxon, the convexity criterion would be violated because the hypothetical ancestors of C, D, and F are in the group (A, B, E). The same is true if F and G are classified together in the same taxon. C and D can be placed in a separate taxon and F and G must be classified as two monotypic taxa of the same rank as (A, B, E). It is clear, however, that if this method is adhered to, the phylogenetic tree cannot be reconstructed from the classification, and cladogenetic information is lost.

Cladists maintain that classifications cannot represent both cladogenetic *and* anagenetic information without becoming ambiguous (Farris, 1976). Furthermore, they claim that a phylogenetic tree is, by its very hierarchical nature, best equipped to serve as the basis for a classification constructed according to the Linnaean system. However, as Phillips (1983) states, "the

Figure 7.2 Illustration of Estabrook's (1978) convexity criterion. By this criterion, *A*, *B*, and *E* are considered to be an acceptable paraphyletic taxon, because the hypothetical ancestors of all inclusive taxa (HA_1, HA_2, and HA_3) are included in the convex path (indicated by the dashed lines).



recognition of paraphyletic taxa remains an attractive option for many taxonomists". I believe Phillips is wrong, however, when he states that "this attraction is simply a consequence of the intuitively observed patterns of overall similarity". While the codification of anagenetic information remains a primary impetus for constructing *evolutionary* classifications (sensu Mayr,1982), paraphyletic classifications remain an attractive option for another reason - the ubiquity of potentially unreliable characters and a lack of sufficient information in many published taxonomic analyses. As with the pronocephalid character-taxon data described in the last chapter, the likely result of insufficient information is the instability of certain regions of the resultant phylogenetic tree.

Phillips (1983:269), in fact, recognises this, for he recommends the following:

> "If only a part of the cladistic history, such as some main lineages, can be determined with confidence, then taxa should be monophyletic as far as possible. For examply, several clearly monophyletic groups may be evident but the confident resolution of clades at the base of the tree is not possible with the available information. A paraphyletic group may be recognised for these taxa, with appropriate notation in the classification indicating its states, pending additional study."

This accurately describes the phylogenetic hypothesis of the pronocephalids given in the Chapter 6, and I believe that Phillips's recommendation is sensible. In this chapter, I describe the protocol used for defining genera of Pronocephalidae on the basis of the species phylogeny. The protocol is based on two criteria founded on courses of action suggested both by Phillips (1983) and Estabrook (1978) .

METHODS

The first criterion relates to the translation of a phylogenetic tree into a classification with paraphyletic groups, and is a modification of the convexity criterion proposed by Estabrook (1978).

Criterion 1

A paraphyletic classification is acceptable if a paraphyletic "super"-taxon is convex, and includes only monophyletic clades of the same cladistic rank as included monophyletic clades of other super-taxa.

I introduce the term *cladistic rank* here, and define it as follows: two monophyletic groups have the same cladistic rank if and only if they are both sub-clades of the same major clade. The clades (A), (B,C,D), (E,F), and (G) are of the same *cladistic* rank. However, (B) and (E), for instance, are not of the same cladistic rank, although according to the conventions of "phyletic sequencing" (Chapter I), they may be accorded the same *taxonomic* rank.

To illustrate the application of Criterion 1, consider Fig. 7.2 again. If (A) is to form a super-taxon, any other paraphyletic super-taxa must be convex groupings of clades of the same phyletic rank. The super-taxa (A), (B,C,D,E,F), and (G) are acceptable but (A), (B), (C,D), (F,E),and (G) are not. This is because the groups (B) and (C,D) are not composed of clades of the same rank as (A), (F,E) and (G). As a futher illustration of the modified convexity criterion, the super-taxa listed in the following classifications are considered acceptable:

Classification 1

(A)

(B,C,D,E,F)

(G)

Classification 2

(A,B,C,D)

(E,F)

(G)

Classification 3

(A,B,C,D)

(E,F,G)

Obviously there are many different ways to classify paraphyletic taxa. In general, a paraphyletic classification will not allow the retrieval of a phylogenetic tree consisting of exactly the same taxonomic units that were used to construct the tree originally. This means that information about the monophyly of super-taxa comprising these taxonomic units is lost. However, using the modified convexity criterion, it is possible to construct paraphyletic classifications so that information about the monophyly of *groups of super-taxa* is still retrievable. I will illustrate this by example.

Consider the following classifications (based on the tree given in Fig. 7.2) which apply the unmodified and modified convexity criteria, respectively:

Classification 1 (unmodified convexity criterion)

(A,B,E)

(C,D)

(F)

(G)

Classification 2(modified convexity criterion)

(A)

(B,C,D,E,F)

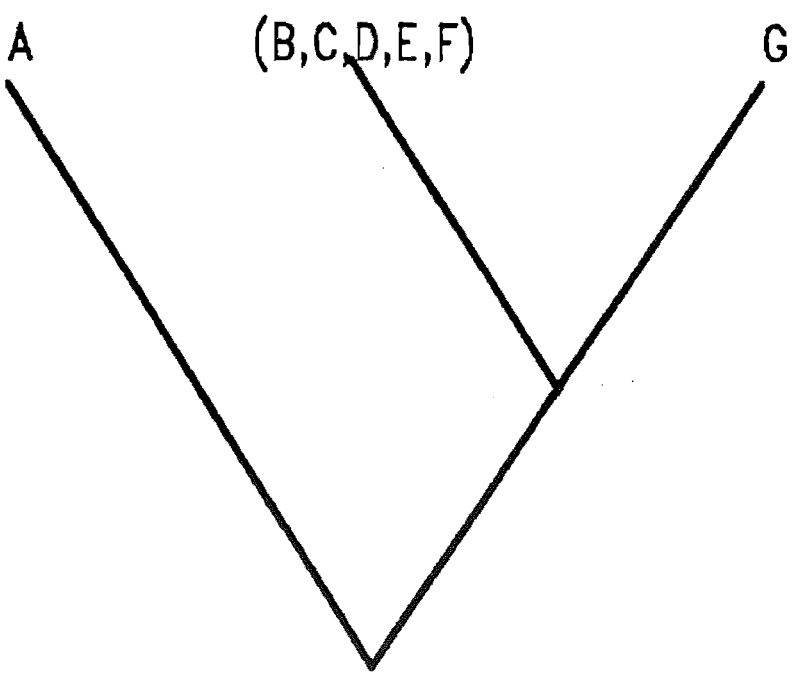
(G)

In the case of Classification 1, there is no way that the paraphyletic group (A,B,E) can be added to any other super-taxon to give a group of super-taxa which is monophyletic with respect to all member taxa. In Classification 2, however, the association of (B,C,D,E,F) and (G) forms a monophyletic group, and therefore some information about monophyly is preserved in this classification. Note, however, that one cannot tell which member of (B,C,D,E,F) is most closely related to (G). Thus the monophyletic information retained pertains only to monophyly of *groups* of super-taxa.

If Classification 2 were a phyletic-sequence representation of a phylogenetic tree, the tree would look like the one given in Fig. 7.3. Clearly, the tree in Fig. 7.3 is simply a "condensing" of the tree in Fig. 7.2. In other words, the relative order of the clades is preserved: (G) is still depicted as being more closely related (i.e., sharing a more recent common ancestor) to (B,C,D,E,F,G) than to (A). Therefore, in addition to preserving some monophyletic information, a paraphyletic classification erected using the modified convex criterion can be used to reconstruct a phylogenetic tree which depicts this information, and the tree itself may be translated into a classification using the phyletic sequencing protocol.

Returning to the recommendation of Phillips quoted at the beginning of this chapter, it is desirable to use paraphyletic groups only when the monophyletic groups are not "clearly" defined. Taxa that are monophyletic because they share a single "questionable" or unstable synapomorphy can probably be included with other groups to form a paraphyletic assemblage. However, how can a decision be made about which groups should be combined, and which should remain as separate (monophyletic) taxa? A second criterion was developed as an operational solution to this problem.

Figure 7.3 A "condensed" classification of the tree given in Fig. 7.2. Such a classification preserves the relative ordering of the super-taxa.



Criterion 2

A classification is acceptable if and only if a systematist can construct a phylogenetic tree of super-taxa (based on the descriptions of these groups and using the same methods as used in the original analysis) which preserves the phylogenetic order of the super-taxa in the way described above.

The value of Criterion 2 can be understood by the following example. Suppose that in Fig. 7.2, (E,F,G) is defined by one "good" synapomorphy, i.e., one that is weighted highly, or one for which there is no missing information among taxa, and has been found to be uniquely derived. The other clades [(A) and (B,C,D)] are defined by "unstable" or "questionable" synapomorphies. Suppose a systematist decides to classify the phylogenetic tree in Fig. 7.2 according to the following system:

(A)
(B,C,D)
(E,F)
(G)

Suppose also that the systematist now attempts a phylogenetic analysis on these super-taxa, describing each super-taxon on the basis of character states that were used in the original analysis and which are found in the majority of taxa in the super-taxon. Since (A) and (B,C,D) are defined by "poor" synapomorphies, it is quite possible that the relative positions of the two groups will be unstable. Therefore, the order in which they appear on a *super-taxon* phylogenetic tree will not necessarily correspond with the order in which they appear in the phylogenetic tree resulting from the original analysis.

Suppose, however, that the systematist decides to classify the group in the following manner:

(A,B,C,D)
(E,F)
(G)

Because the clade consisting of the groups (E,F) and (G) is defined by a "good" synapomorphy, this classification will retain the relative order of the clades, subject to the "condensing" of clades (A) and (B,C,D).

As a final illustration, suppose the systematist erects the following classification of A to G:

(A)
(B,C,D,E,F)
(G)

Since the clade (E,F) is now subsumed under the larger paraphyletic group (B,C,D,E,F), the "good" synapomorphy separating (E,F,G) cannot be recovered from the data set. As in the first example, a phylogenetic analysis of these super-taxa may not reflect the order of the original tree.

For Criterion 2 to be satisfied, monophyletic groups defined by "good" synapomorphies should not be added to paraphyletic assemblages, and paraphyletic groups should include as many "poorly-defined" clades as possible. Therefore, to find an acceptable classification, it is only necessary to reconstruct phylogenetic trees for different paraphyletic classifications, and choose the classification which preserves the relative cladistic order of the original tree. Criterion 2 frees the systematist from having to decide which groups are defined by "good" synapomorphies and which are defined by "poor" synapomorphies.

Criteria 1 and 2 were applied to construct a classification of the Pronocephalidae based on its phylogeny. The following protocol was used:

1. Three different classifications of the Pronocephalidae, each derived from the phylogenetic tree given in Fig. 6.11, were constructed (Table 7.1). The first classification was strictly monophyletic, whereas the other three were paraphyletic.
2. A composite character state vector was defined for each super-taxon by taking, for each character, the state that occurred in at least 75% of the taxa in the super-taxon (Table 7.1). For any super-taxon, if a character was polymorphic and the dominant state was found in less than 75% of the member-taxa, the character was coded "9" (for unknown or missing information).
3. PAUP was used to conduct a weighted parsimony analysis on each data set, using the same weights as in the original analysis. The resultant trees are presented in Fig. 7.4.

RESULTS AND DISCUSSION

The first and third data sets resulted in phylogenies in which the super-taxa were in a different cladistic order to that of the original tree (Fig. 7.4a & c). Classification 2 is the only scheme in which the cladistic order of the hypothesised phylogeny of the Pronocephalidae is preserved. Therefore, it forms the basis of the classification presented in the next chapter.

Table 7.1 Three character-supertaxa datasets for the Pronocephalidae. The names of the supertaxa correspond to the clades illustrated in Fig. 6.11. Dataset (A) consists of only monophyletic groups [Note: some species have been excluded from (A) (e.g., *Pleurogonius americanus*), and would be treated as *species incertae sedis* should the classification be acceptable]. (B) and (C) have paraphyletic supertaxa.

Rhabdiopoeius	0 0 0 0 1 0 1 0 0 0 5 9 0 9 0 0 0 1 2 0 0 2 1 0 0 1 1 0 1 1 0 0 1 4 0 0 1 0 0 2 1 9 0 1 0 0 0 0 0 0
I	0 9 9 9 1 0 9 0 9 0 1 9 1 0 0 0 0 0 2 2 0 9 0 9 0 1 1 0 0 1 9 0 1 1 0 0 9 0 0 9 1 9 0 1 0 0 0 0 0 0
H	1 9 0 0 1 0 1 0 9 0 1 0 0 0 0 9 0 1 1 9 0 0 0 9 0 0 0 0 0 0 1 1 0 9 1 0 0 0 0 0 2 1 1 0 1 1 0 0 0 0 0
G(b)	1 1 9 9 1 0 1 0 1 0 1 9 1 9 0 0 0 1 1 9 0 9 9 9 9 0 0 0 0 1 9 9 9 9 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
F	9 9 9 9 1 9 1 0 9 0 9 9 9 9 0 0 9 9 1 9 0 0 0 9 9 0 9 9 0 1 9 1 9 9 0 9 9 0 0 2 1 1 0 3 0 0 9 9 9 0
E	1 9 9 9 1 0 1 0 9 0 2 1 0 9 0 0 9 9 1 9 0 9 9 1 0 9 9 0 0 1 9 0 1 9 0 0 9 0 0 2 1 1 0 1 1 0 0 0 0 0
G(c)	1 2 9 9 1 1 1 9 3 0 1 0 0 3 0 0 0 1 1 0 0 9 0 1 9 0 9 9 0 0 9 0 1 9 0 0 0 0 0 2 1 1 0 1 0 0 0 0 0 0
G(a)	1 9 9 1 1 9 1 0 1 0 1 9 0 9 0 0 0 1 1 9 9 9 9 9 9 0 9 9 0 1 0 1 9 2 0 9 9 0 1 2 1 9 0 1 0 0 0 0 0 0
Notocotyloides petasatum	1 3 1 2 1 0 1 0 0 0 1 0 0 3 0 0 0 0 2 2 0 0 0 0 0 0 1 0 9 9 9 9 1 1 0 1 9 0 0 2 1 3 0 1 0 0 0 0 0 0
D	0 0 0 0 2 0 1 0 0 2 2 1 0 1 1 1 2 0 1 9 9 9 0 1 9 0 9 0 0 1 1 0 9 9 0 1 1 0 0 2 3 0 0 1 1 0 0 0 0 0
C	1 2 1 1 1 0 1 0 0 2 2 1 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 9 0 1 0 0 0 0 1 0 2 2 0 1 1 1 0 0 0 0 0
B	1 2 9 0 1 9 1 9 0 2 1 9 0 9 0 9 0 1 1 0 9 2 1 1 0 0 9 1 0 1 9 9 0 9 2 0 0 0 0 2 9 0 1 1 0 1 0 0 0 1
G(d) + G(e) + G(f)	9 9 9 9 1 0 1 0 9 0 1 9 9 9 9 0 0 1 1 9 9 9 9 9 9 9 9 9 9 1 9 9 9 9 0 9 9 0 0 9 1 9 0 1 0 0 0 0 0 0

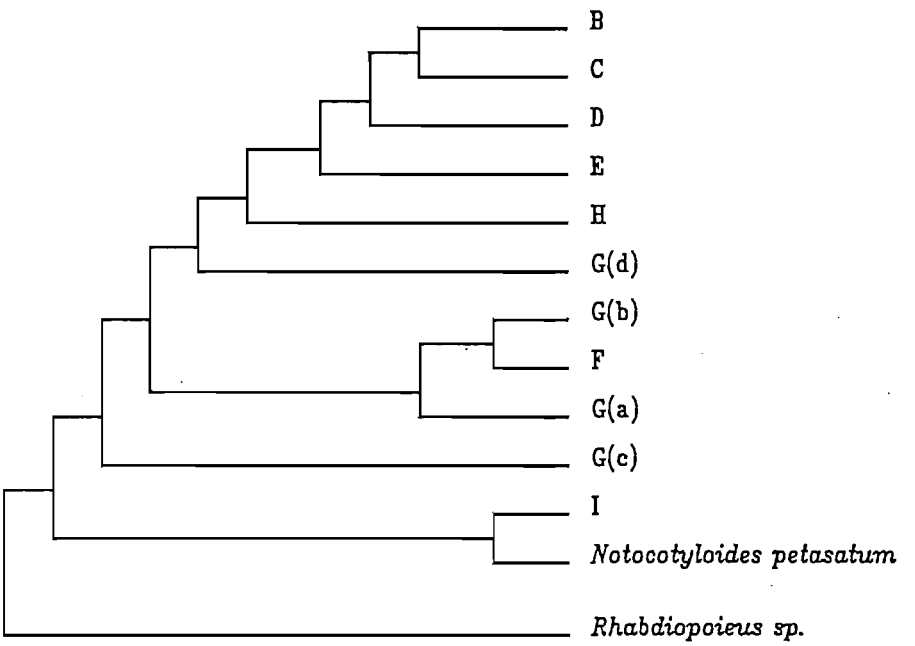
Rhabdiopoeius	0 0 0 0 1 0 1 0 0 0 5 9 0 9 0 0 0 1 2 0 0 2 1 0 0 1 1 0 1 1 0 0 1 4 0 0 1 0 0 2 1 9 0 1 0 0 0 0 0 0
I	0 9 9 9 1 0 9 0 9 0 1 9 1 0 0 0 0 0 2 2 0 9 0 9 0 1 1 0 0 1 9 0 1 1 0 0 9 0 0 9 1 9 0 1 0 0 0 0 0 0
F	9 9 9 9 1 9 1 0 9 0 9 9 9 9 0 0 9 9 1 9 0 0 0 9 9 0 9 9 0 1 9 1 9 9 0 9 9 0 0 2 1 1 0 3 0 0 9 9 9 0
E	1 9 9 9 1 0 1 0 9 0 2 1 0 9 0 0 9 9 1 9 0 9 9 1 0 9 9 0 0 1 9 0 1 9 0 0 9 0 0 2 1 1 0 1 1 0 0 0 0 0
G + H	9 9 9 9 1 9 1 9 9 0 1 9 9 9 9 9 0 1 1 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 0 9 9 0 9 9 1 9 0 1 9 0 0 0 0 0
Notocotyloides petasatum	1 3 1 2 1 0 1 0 0 0 1 0 0 3 0 0 0 0 2 2 0 0 0 0 0 0 1 0 9 9 9 9 1 1 0 1 9 0 0 2 1 3 0 1 0 0 0 0 0 0
D	0 0 0 0 2 0 1 0 0 2 2 1 0 1 1 1 2 0 1 9 9 9 0 1 9 0 9 0 0 1 1 0 9 9 0 1 1 0 0 2 3 0 0 1 1 0 0 0 0 0
C	1 2 1 1 1 0 1 0 0 2 2 1 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 1 9 0 1 0 0 0 0 1 0 2 2 0 1 1 1 0 0 0 0 0
B	1 2 9 0 1 9 1 9 0 2 1 9 0 9 0 9 0 1 1 0 9 2 1 1 0 0 9 1 0 1 9 9 0 9 2 0 0 0 0 2 9 0 1 1 0 1 0 0 0 1

Rhabdiopoeius	0 0 0 0 1 0 1 0 0 0 5 9 0 9 0 0 0 1 2 0 0 2 1 0 0 1 1 0 1 1 0 0 1 4 0 0 1 0 0 2 1 9 0 1 0 0 0 0 0 0
G+H+Notocotyloides petasatum	9 9 9 9 1 9 9 9 9 9 1 9 9 9 0 9 0 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 0 9 9 0 9 9 1 9 0 1 9 0 0 0 0 0
F	9 9 9 9 1 9 1 0 9 0 9 9 9 9 0 9 9 9 1 9 0 9 0 9 9 9 9 9 9 0 1 9 1 9 9 0 9 9 0 0 2 1 1 0 3 0 0 9 9 9 0
E	1 9 9 9 1 0 1 0 9 0 2 1 0 9 0 0 9 9 1 9 0 9 9 1 0 9 9 0 0 1 9 0 1 9 0 0 9 0 0 2 1 1 0 1 1 0 0 0 0 0
D	9 9 9 0 2 0 1 0 0 2 2 1 0 9 1 1 2 9 1 9 9 9 9 9 9 9 9 0 9 9 1 9 9 9 9 9 9 9 0 2 3 0 0 1 1 0 0 0 0 0
C	1 2 9 1 1 9 1 0 0 2 2 1 0 0 0 1 1 0 1 9 0 0 0 0 0 0 0 9 0 1 9 9 9 0 0 9 0 1 0 2 2 0 1 1 1 0 0 0 0 0
B	1 2 9 0 1 9 1 9 9 2 1 9 0 9 0 9 0 1 1 0 9 2 1 9 0 0 9 1 0 9 9 9 0 9 9 9 9 0 0 0 2 9 0 1 1 0 9 0 0 0 1

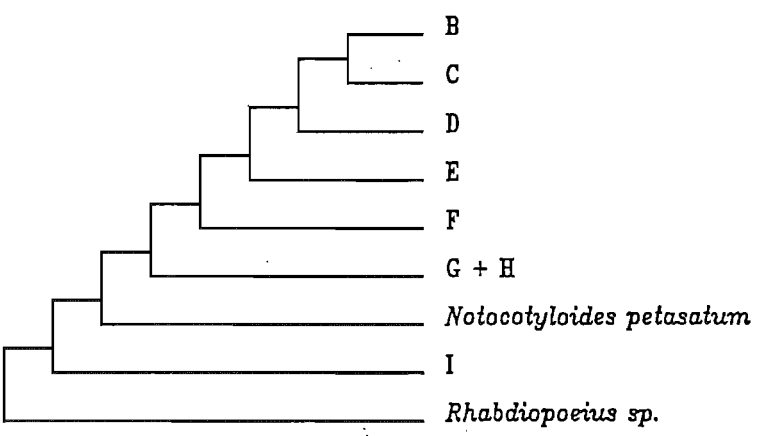
C

Figure 7.4 Resultant trees of phylogenetic analyses on the datasets given in Table 7.1. Only (B) preserves the relative cladistic order of the phylogeny of the Pronocephalidae.

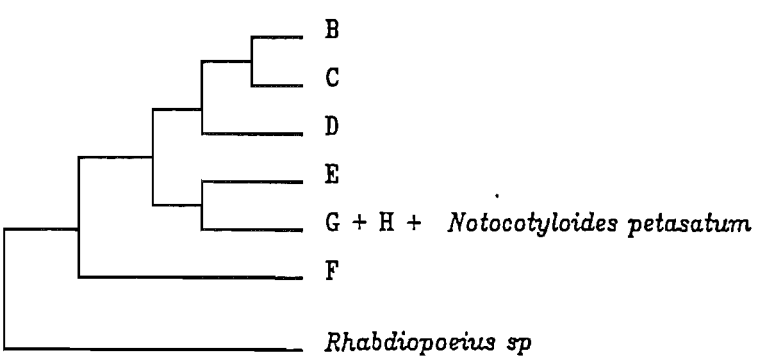
A



B



C



CHAPTER 8

A TAXONOMIC REVISION OF THE PRONOCEPHALIDAE LOOSS, 1902 (PLATYHELMINTHES : DIGENEA)

INTRODUCTION

In this chapter, a classification of the Pronocephalidae Looss, 1902, (Platyhelminthes: Digenea) is presented, based on the phylogenetic hypothesis discussed in Chapter 6. Details of the procedure used to translate the phylogenetic hypothesis into a classification are given in Chapter 7.

CONVENTIONS

The classification follows the convention of phyletic sequencing detailed by Nelson (1978) and Wiley (1981). The order in which taxa of the same rank are listed and described reflect the order in which they appear on the phylogenetic tree (Fig. 7.4b). Taxa hypothesised to have arisen earlier are listed first.

If a genus is paraphyletic, a comment to that effect is made in the diagnosis. Within a paraphyletic genus, species that form monophyletic assemblages and which were previously assigned to a (now synonymised) genus, are placed in informal species-groups. These species-groups are referred to using the superseded generic name in quotes. Member species of each genus are listed in Table 8.1.

In the classification, genera are not assigned to sub-families because the phylogenetic tree of pronocephalid genera is completely asymmetrical (Fig. 7.4b). Consequently, sub-families would, by necessity, have to be monogeneric, or if polygeneric, must include all genera in order to satisfy the condition of monophyly. Since monogeneric sub-families and those which include all genera are taxonomically redundant (Wiley, 1981), sub-family designations are not used. However, I will discuss the validity of sub-families erected by previous authors with respect to their monophyly, when the relevant genera are discussed.

CLASSIFICATION

PRONOCEPHALIDAE Looss, 1902

Synonyms. Pronocephalinae Looss, 1899
Opisthoporidae Fukui, 1929
Metacetabulidae Frietas and Lent, 1936
Neopronocephalidae Groschaft and Tenora, 1981
Charaxicephalidae Groschaft and Tenora, 1981

Diagnosis (modified after Yamaguti, 1971).

Monostomatous Digenea usually with more or less elongate, canoe- or spoon-shaped body, sometimes vermiform. Ventral glands usually absent. Setose or smooth and invaginated papillose processes sometimes present at posterio-lateral margins of body. Cephalic musculature often developed into collar-like ridges or lateral lobes; sometimes undeveloped. Oral sucker

Figure 8.1 Genera and species of the Pronocephalidae Looss, 1902.

Footnotes:

a This genus is paraphyletic.

b The specific name of the original taxon (*Rameshwarotrema chelonei* Lakshman Rao, 1975) is a secondary homonym of *Pleurogonius chelonei* (Chattopadhyaya, 1972). A new nominal taxon is therefore erected to accomodate the species.

c The specific name of the original taxon (*Desmogonius loossi* Chattopadhyaya, 1972) is a secondary homonym of *Charaxicephalus loossi* Mehra, 1932. A new nominal taxon is therefore erected to accomodate the species.

FAMILY Pronocephalidae Looss, 1902

GENUS *Notocotyloides* Dollfus, 1966
N. petasatum (Deslongchamps) Dollfus, 1966

GENUS *Pyelosomum* Looss, 1899[a]
P. americanum (Caballero, Zereco and Grocotti, 1955)
P. cheloniae (Oguro, 1936)
P. macrophallus (Oguro, 1936)
P. solidum (Looss, 1901)
P. keamarii (Mehra, 1939)
P. carettae (Chattopadhyaya, 1976)
P. candidulum (Linton, 1910)
P. longiusculum (Looss, 1901)
P. vitallani (Gupta, 1962)
P. trigonocephalum (Rudolphi, 1809)

"Adenogaster"-group
P. serialis (Looss, 1901)
P. ocadiae (Oshmarin and Zharikova, 1984)
P. indica (Lakshman Rao, 1975)

P. grocotti (Caballero, 1954)
P. karachii (Mehra, 1939)
P. raoensis nomen novum[b]
P. uterocrescens (Lakshman Rao, 1975)
P. bilobum (Looss, 1901)
P. mandapamensis (Chattopadhyaya, 1972)
P. brevicaecum (Sullivan, 1976)
P. cuorensis (Brooks and Palmieri, 1978)
P. orientalis (Mehra, 1939)
P. holacanthi (Siddiqi and Cable, 1960)
P. erubescens (Linton, 1910)
P. malaclemys (Hunter, 1961)
P. linearis (Looss, 1901)
P. truncatum (Prudhoe, 1944)
P. laterouterus (Fischtal and Acholonu, 1976)
P. latum (Fischtal and Acholonu, 1976)
P. puertoricensis (Fischtal and Acholonu, 1976)
P. ozakii (Oguro, 1936)
P. minutissimum (Looss, 1901)

"Cricocephalus"-group
P. megastomum (Looss, 1902)
P. resectum (Looss, 1902)
P. indicum (Chattopadhyaya, 1972)
P. koidzumii (Kobayashi, 1921)
P. album (Kuhl and Hasselt, 1823)

P. stenobursata (Fischthal and Acholonu, 1976)
P. mcintoshi (Siddiqi and Cable, 1960)
P. sindhii (Mehra, 1939)
P. crassa (Looss, 1901)

P. navicularis (Gilbert, 1938)
P. chelonii (Mehra, 1939)
P. lobatum (Looss, 1901)

"Myosaccus"-group
P. longicaecum Luhman, 1935
P. posterorchis Oguro, 1936
P. renicapite (Leidy, 1856)
P. cochlear Looss, 1899
P. amblyrhynchi (Gilbert, 1938)
P. parvum Prudhoe, 1944
P. chelonei (Chattopadhyaya, 1972)

GENUS *Charaxicephalus* Looss 1901
C. loossi Mehra, 1929
C. robustus Looss, 1901
C. polyorchis (Groschaft and Tenora, 1978)
C. desmogonius (Stephens, 1911)
C. sinistroporus nomen novum[c]
C. lateralis (Oguro, 1936)
C. prafullai (Chattopadhyaya, 1972)
C. ellipticus (Pratt, 1914)
C. pandus (Braun, 1901)
C. singhi (Lakshman Rao, 1975)
C. takahashii (Fukui and Ogata, 1936)
C. multitesticularis (Rohde, 1962)
C. kachugai (Lakshman Rao, 1975)
C. gastricus (Mehra, 1932)

GENUS *Pronocephalus* Looss, 1901
P. mehrai Chattopadhyaya, 1972
P. obliquus Looss, 1901
P. aspidonectes (Fukui, 1929)

GENUS *Cetiosaccus* Gilbert, 1938
C. galapagensis Gilbert, 1938
C. invaginatus (Frietas and Lent, 1938)
C. karachiense (Bilqees, 1974)
C. yamagutii (Chattopadhyaya, 1972)

GENUS *Macravestibulum* Mackim, 1930
M. kepneri Jones, Mounts, and Wollcott 1942
M. eversum Hsu, 1937
M. kraatzi Damian, 1961
M. obtusicaudatum Mackim, 1930

GENUS *Neopronocephalus* Mehra, 1932
N. ocellata Dwivedi, 1977
N. spinosa Dwivedi, 1977
N. mehrai Chatterji, 1936
N. orientalis Brooks and Palmieri, 1979
N. kachugai Jahan, 1970
N. spinometratermis Lakshman Rao, 1975
N. triangularis Mehra, 1932

simple, terminal or sub-terminal. Pharynx absent, although a small bulbous swelling may be present at posterior end of oesophagus. Caeca usually parallel with lateral margins of body, sometimes sinuous; diverticulate, or with smooth or crenated lateral margins; terminating at or near posterior extremity of body, or some distance short of it. Testes with smooth margins, lobed, or follicular; bilaterally placed, oblique or tandem, with centres below level of ovary, exceptionally pre-ovarian. Cirrus sac enclosing ejaculatory duct, prostate, and sometimes part of seminal vesicle; ejaculatory duct rarely absent. Genital pore always pre-equatorial, usually sub-median and below level of caecal bifurcation. Male and female pores close together, separate or opening into a shallow genital atrium. Ovary in posterior third of body, seminal receptacle rarely present. Mehlis' gland generally posterior to or at level with ovary; very rarely anterior to ovary. Vitellaria acinous or follicular, in two lateral fields, usually extracaecal and postovarian. Uterus usually coiled transversely anterior to ovary, exceptionally extending between the two testes and behind the ovary. Eggs small, often with polar filaments, generally unioperculate, exceptionally bioperculate or non-operculate. Excretory vessels simple, diverticulate or with anastomoses; meeting simply or in network at region of oesophagus, or terminating blindly and not not uniting. Excretory vesicle small and Y- or V-shaped, or voluminous. Excretory bladder or vessels sometimes emptying into vestibular cavity. Excretory pore dorsal or terminal, exceptionally ventral, at posterior extremity. Parasitic in digestive tract of marine and freshwater turtles, exceptionally in marine iguanids, fishes and birds.

Type genus: *Pronocephalus* Looss, 1899

Other genera: *Notocotyloides* Dollfus, 1966

Pyelosomum Looss, 1899

Charaxicephalus Looss, 1901

Cetiosaccus Gilbert, 1938

Macravestibulum Mackim, 1930

Neopronocephalus Mehra, 1932

Genera incertae sedis:

Choanophorus Caballero, 1942

Ruicephalus (Ruiz, 1946) Skrjabin, 1955

Discussion

Ruiz (1946) included members of the Notocotylidae Luhe, 1909, in the sub-family Notocotylinae Kossack, 1911, of the family Pronocephalidae. Ruiz also included two other sub-families within the Pronocephalidae: the Nudacotylinae Barker, 1916, and the Opisthotrematinae Harwood, 1939. These groups are considered to be less closely related to the Pronocephalidae than are the notocotylids and therefore they were ommitted from the phylogenetic analysis.

Unfortunately, a phylogenetic hypothesis offers no way of solving the dilemma of taxonomic rank. It is clear from the phylogeny given in Fig. 6.13 that the Notocotylidae is closely related to the Pronocephalidae, and Ruiz's (1946) revision is not incompatible with this phylogeny. Nevertheless, I have chosen to retain the pronocephalids and notocotylids as separate families, in accordance with the widely accepted convention.

Another interesting problem concerns the taxonomic position of the sub-family Parapronocephalinae which Sinclair (1972) erected the to contain the genus *Parapronocephalum* Belopolskaia, 1952. He placed the sub-family in the Notocotylidae because of the presence of ventral glands in the type and only contained genus. However, *Parapronocephalum* is characterised by a collar-like modification of the cephalic region, and has been thought of as a "link" between the notocotylids and pronocephalids (Sinclair, 1972) [the genus has been placed in the Pronocephalinae of the Pronocephalidae by Yamaguti (1958) and Skrjabin (1955)]. Yamaguti (1972) also added the genus *Notocotyloides* Dollfus, 1966 to the Parapronocephalinae, and although he makes no mention of this, one assumes that it was because of its resemblance to *Parapronocephalum* from which it differs only in the absence of ventral glands. He also transferred the sub-family Parapronocephalinae to the Pronocephalidae, although again he gave no justification for this.

According to my phylogeny of the Pronocephalidae, any taxonomic association of *Parapronocephalum* and *Notocotyloides* must be paraphyletic. *Parapronocephalum* is unequivocally part of the "notocotylid" group. Therefore, it is *Notocotyloides* which seems to be the species intermediate between the notocotylids and the pronocephalids. In this classification, only *Notocotyloides* is included in the Pronocephalidae.

Other reviewers have added or removed genera from the Pronocephalidae at various times, and sometimes erected or synonymised families to do so (e.g.,Groschaft and Tenora, 1981; Yamaguti, 1958). The validity of these changes is discussed when reference is made to the

appropriate genera.

Key to genera:

1. Ovary posterior to Mehlis' gland .....*Notocotyloides*
- Ovary anterior to or at level with Mehlis' gland 2
2. Testes follicular .....*Charaxicephalus*
- Testes whole 3
3. Testes completely preovarian .....*Neopronocephalus*
- Testes largely postovarian 4
4. Vestibule present .....*Macravestibulum*
- Vestibule absent 5
5. Excretory bladder present and voluminous.....*Cetiosaccus*
- Excretory vesicle simple, not well-developed 6
6. Testes intercaecal .....*Pronocephalus*
- Testes ventral to caeca or extracaecal .....*Pyelosomum*

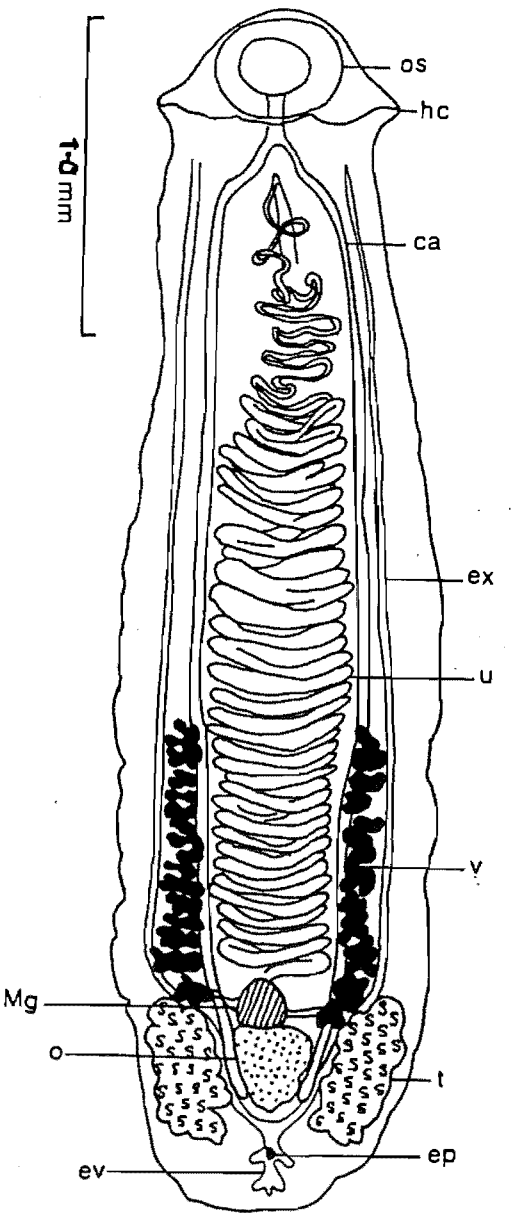
Notocotyloides Dollfus, 1966 (Fig. 8.1)

Diagnosis (modified after Yamaguti, 1972)

Pronocephalidae. Body elongate, flat, somewhat enlarged posteriorly with somewhat crenulated margins. No dermal glands. Head collar projecting laterad prominently, assuming the appearance referring to as "petasatum". Oral sucker large, oesophagus short; caeca with smooth lateral margins, converging mediad. Testes slightly lobed, symmetrical, extracaecal, lateral to ovary, at posterior of body. Cirrus pouch apparently elongate. Genital pore median, below caecal bifurcation. Ovary slightly lobed, in median position, below Mehlis' gland. Vitellaria in to lateral linear fields, not extending to equator. Uterus in transverse intercaecal coils. Eggs small, presence or absence of polar filaments unknown. Excretory vesicle Y-shaped, digitiform. Pore ventral, close to extreme posterior end of body. Parasites of birds.

Type and only species: *N. petasatum* (Deslongchamps, 1824) Dollfus, 1966; syn. *Monostoma petasatum* D.

Figure 8.1 *Notocotyloides petasatum* Dollfus, 1966 (after Dollfus, 1966). ca = caecum; ep = excretory pore; ev = excretory vessel; hc = head collar; Mg = Mehlis' gland; o = ovary; os = oral sucker; t = testes; u = uterus; v = vitellaria.



Discussion

As stated above, the monospecific genus *Notocotyloides* bears a structural resemblance to *Parapronocephalum reversum.* However, as Groschaft and Tenora (1981) point out, *P. reversum*, unlike *P. symmetricum* Belopolskaia, 1952, has extracaecal testes, whereas in the latter the testes are intercaecal. Groschaft and Tenora (1981) also considered that the presence or absence of ventral glands in *N. petasatum* was uncertain, either because the worms studied were sexually immature, or the stain used obscured them. However, Dollfus's (1966) illustration of *N. petasatum* shows well developed vitellaria, ovary, and testes, suggesting that the animals were indeed sexually mature. Dollfus offers no information regarding the staining technique. Groschaft and Tenora (1981) regard *P. reversum* as a *species incertae sedis*, because of its similarity to *N. petasatum*, and because of the "uncertainty" regarding the presence of ventral glands in the latter species.

In this analysis, Dollfus's (1966) description was taken as correct and the absence of ventral glands is used as a feature of *N. petasatum.* As stated earlier, the genus *Parapronocephalum* was represented solely by *P. reversum* because of a lack of pertinent information regarding the morphology of *P. symmetricum.* However, to reiterate, the phylogenetic hypothesis presented here does not support the association of *P. reversum* and *N. petasatum* as a monophyletic group: the genus *Notocotyloides* is part of the clade which includes the other species of the Pronocephalidae, whereas *Parapronocephalum* (represented in this analysis by *P. reversum*) is part of the Notocotylidae.

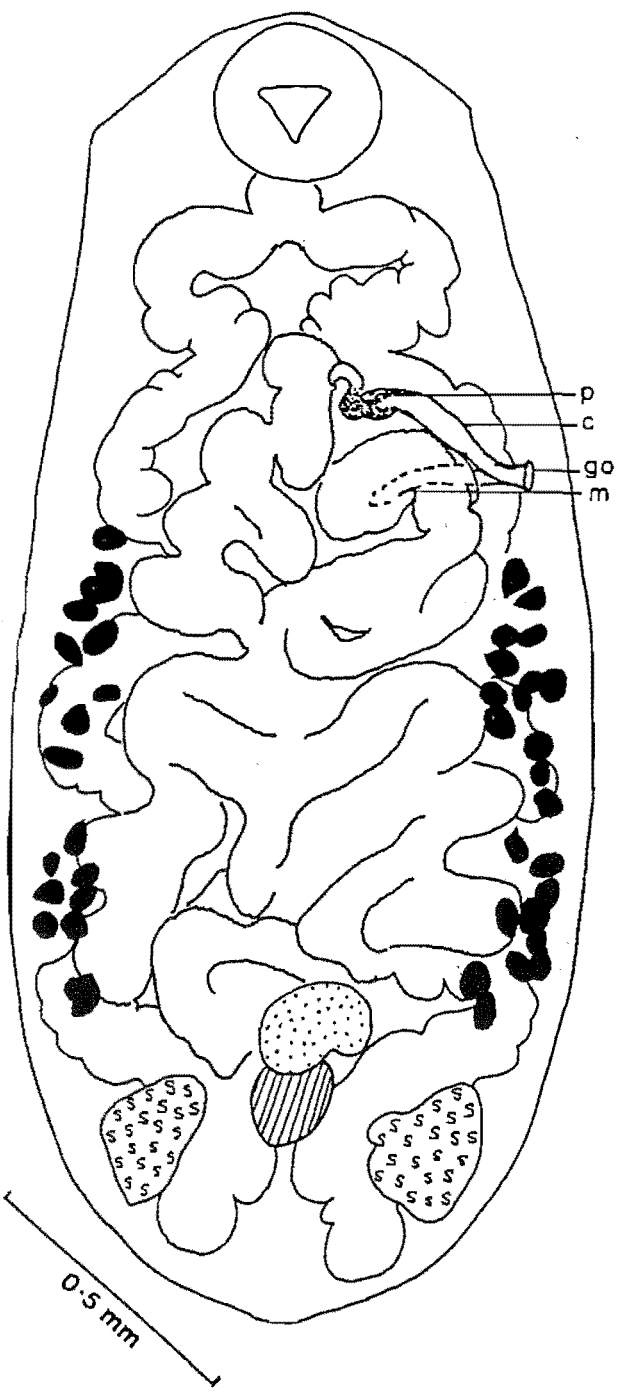
Pyelosomum Looss, 1899 (Fig. 8.2)

Synonyms *Cricocephalus* Looss, 1899
Adenogaster Looss, 1901
Epibathra Looss, 1901
Glyphicephalus Looss, 1901
Pleurogonius Looss, 1901
Barisomum Linton, 1910
Himasomum Linton, 1910
Astrorchis Poche, 1926
Medioporus Oguro, 1936
Myosaccus Gilbert, 1938
Iguanacola Gilbert, 1938
Renigonius Mehra, 1939
Pseudobarisomum Siddiqi and Cable, 1960
Neocricocephalus Gupta, 1962
Rameshwarotrema Lakshman Rao, 1975
Parapleurogonius Sullivan, 1976
Cortinasoma Oshmarin and Zharikova, 1984
Raogaster Groschaft and Tenora, 1981

Emended diagnosis

Pronocephalidae (paraphyletic). Body elliptical or elongate, usually canoe-shaped. Cephalic musculature in form of collar-like ridge or lateral lobes, sometimes weakly developed or

Figure 8.2 *Pyelosomum cochlear* Looss, 1899 (after Caballero, Zerecero, and Grocott, 1955). c = cirrus complex; go = genital opening; m = metraterm; p = pars prostatica.



absent. Posterior papillate processes present or absent. Ventral glands present or absent. Oral sucker terminal or sub-terminal. Caeca with diverticulate, or smooth or crenated margins; sometimes sinusoidal, but mainly running parallel with lateral margins of body; extending to posterior of body and converging mediad; sometimes extending only to anterior margins of testes. Simple excretory vesicle present, Y- or V-shaped. Excretory vessels diverticulate or anastomosing, terminating blindly at anterior of body, or uniting anterior to caecal bifurcation. Excretory pore almost always dorsal. Testes whole, lobed, or with smooth margins, usually extracaecal, never intercaecal, with centres always post-ovarian. Cirrus sac enclosing ejaculatory duct, prostate, and sometimes internal seminal vesicle; may be bipartite, with posterior portion enclosing prostrate; placed longitudinally, or transversely. Genital openings common or separate, intercaecal, ventral to caeca or extracaecal; uually below level of caecal bifurcation, exceptionally, above caecal bifurcation. Ovary at level with or anterior to Mehlis' gland, median or left of midline. Vitellaria in compact, or linear fields. Uterine coils in orderly transverse loops, inter- or extracaecal, rarely extending to margins of body. Metraterm slender or muscular. Eggs with or without polar filaments. Parasites of marine turtles, marine iguanids, and and and and rarely, marine teleosts.

Type species: *Pyelosomum cochlear* Looss, 1899

Discussion

The genus *Pyelosomum* corresponds loosely to the sub-families Pronocephalinae Looss, 1899 (sensu Yamaguti, 1972) and Pronocephalinae Looss, 1899 (sensu Groschaft and Tenora, 1981). My decision to synonymise 17 pronocephalid genera (which have traditionally been assigned to a sub-family) will certainly be contentious. Therefore, I will explain the reasons for my choice of rank in detail.

First, about a third of the genera listed as synonyms are monotypic genera. In general, monotypic taxa are considered redundant because there is no information gain or utility in erecting two or more taxa, of different ranks, to contain a single group. A case can be made for a monotypic taxon if it can be shown that the rank of the taxon is consistent with the ranks of its sister group. In other words, if other polytypic taxa of a certain rank are sister groups of a monotypic taxon, then the rank of the monotypic

taxon should reflect this. However, the analysis presented here indicates that many of the (now synonymised) polytypic genera were in fact polyphyletic. Consequently, in accordance with the conventions of phylogenetic systematics, and the criteria discussed in Chapter 7, both the ranks and validity of member genera of the Pronocephalinae are inappropriate and require revision.

Another reason for synonymising the genera relates to the phylogenetic hypothesis of the Pronocephalidae and the stability of characters separating the groups (Chapter 7). The lack of character information for many member species of the synonymised genera, coupled with hypotheses of relatively high rates of change for certain characters that had been used to separate groups (e.g. Character 14: nature of the anterior junction of excretory vessels; used by Looss, 1901 to distinguish *Glyphicephalus* from *Pleurogonius*) resulted in unstable assemblages of species (Chapter 6), that did not necessarily correspond to nominal genera. When such assemblages were re-analysed according to the protocol given in Chapter 7, their relative phylogenetic order could only be retrieved when all member species of genera in the synonym list were assigned to a single taxon.

To some extent, the assignment of taxonomic rank above the species level is always a subjective decision. Within the constraints of the criteria discussed in Chapter 7, I have assigned taxonomic ranks so as to reduce the redundancy of the classification of the Pronocephalidae. It should also be noted that other authors have seen the need to synonymise many of the genera listed above. For instance, Price (1931) synonymised *Himasomum* and *Barisomum* with the latter as the nominal taxon. He also argued for the synonymy of *Barisomum*, *Glyphicephalus*, and *Epibathra*. R.K. Mehra (1939) synonymised the genera *Pleurogonius*, *Glyphicephalus*, *Barisomum*, and *Myosaccus* under the nominal genus *Pleurogonius*. His father, H.R. Mehra (1981) added *Medioporus* as a synonym of *Pleurogonius* (sensu R.K. Mehra, 1939). Ruiz (1946) considered *Astrorchis* to be a synonym of *Pyelosomum*. Subsequently, Threlfall (1979) synonymised *Pyelosomum* (sensu Ruiz, 1946), *Epibathra Myosaccus*, *Astrorchis*, and *Pleurogonius* (sensu R.K. Mehra, 1939), under *Pyelosomum*. Threlfall's classification of *Pyelosomum* is closest to the one given here.

Within *Pyelosomum*, certain species groups are defined by characters that are hypothesised to be good indicators of monophyly. Members of these groups have previously been assigned to separate nominal genera. Given the relative constancy of these species groups, I believe it is appropriate to give an additional description of each. The earliest available generic name will

be used as the *informal* group name, for taxonomic convenience.

"Myosaccus"-group

Description

Members of *Pyelosomum*, previously placed by various authors in *Pyelosomum*, *Astrorchis*, and *Myosaccus*. With the variable characteristics of *Pyelosomum* except for the following: Dorsal cephalic ridge present. Ventral glands absent. Caeca with crenated margins, and sinusoidal. Excretory vesicle Y-shaped. Cirrus sac enclosing part of seminal vesicle; not bipartite. Genital openings post-bifurcal.

Discussion

The definitive feature of the group are the sinuous caeca present in all members. This feature is unique to members of the "Myosaccus"-group.

"Cricocephalus"-group

Description

Members of *Pyelosomum*, previously placed in *Cricocephalus* Looss, 1899. Head collar with dorsal, and slightly incised ventral ridges. Dome-shaped or stalked posterior papillate processes always present. Caeca diverticulate, extending to posterior extremity of body, and converging mediad. Excretory vesicle Y-shaped. Excretory vessels not meeting anteriorly. Cirrus sac bipartite, with posterior portion enclosing prostate. Metraterm muscular. Vitellaria in two compact fields. Testes lobed. Eggs unifilamentous at both ends. Parasites of marine turtles.

Discussion

The "Cricocephalus"-group is distinguished from other members of the genus primarily on the basis of the bipartite cirrus sac. Gilbert (1938) stated that this feature was also found in *Pyelosomum navicularis* (Gilbert, 1938) (= *Iguanacola navicularis*) which is not a member of this species group (Table 8.1). However, Gilbert's (1938) illustration of the cirrus sac of *P. navicularis* indicates only a slight constriction between the ejaculatory duct and the prostate, very much like that seen in a number of other *Pyelosomum* species [e.g., *P. brevicaecum* (Sullivan, 1976) (= *Parapleurogonius brevicaecum*)]. The constriction between the two parts of the cirrus sac of the "Cricocephalus"-group is more pronounced and only a slender canal connects the prostate to the ejaculatory duct.

"Adenogaster"-group

Members of *Pyelosomum*, previously placed by other authors in *Adenogaster*, *Cortinasoma*, and *Raogaster*. With variable characteristics of the genus *Pyelosomum* except for the following: Ventral cephalic ridge incised. Posterior papillate processes absent. Ventral glands present in longitudinal rows or irregularly placed on ventral surface. Cirrus not bipartite. Common male and female genital opening, below level of caecal bifurcation. Parasites of aquatic turtles.

Discussion

Almost certainly, the decision to synonymise the genus *Adenogaster* under *Pyelosomum* will not be accepted unequivocally. The "Adenogaster"-group consists of species that are readily defined by a character which has previously been used as a diagnostic feature for families - the presence of ventral papillae or glands. In fact, Groschaft and Tenora (1981) erected the sub-family Adenogasterinae to contain *Adenogaster* and *Raogaster* and considered it to be closely related to the Parapronocephalinae (which contained the genera *Parapronocephalum* and *Notocotyloides*). All four genera were considered to be "intermediate forms" between the notocotylids and the pronocephalids. However, Beverley-Burton (1972), in her study of the ventral papillae of notocotylids, noted that the papillae are structurally variable. Similarly, Sinclair (1972) noted that the ventral glands of *P. reversum* differed from those of notocotylids in shape and structure. There is therefore no real evidence that ventral glands could not have evolved independently in a number of groups. Even ontogenetic evidence does not dispel this possibility, because the glands are not apparent in sexually immature forms (Groschaft and Tenora, 1981).

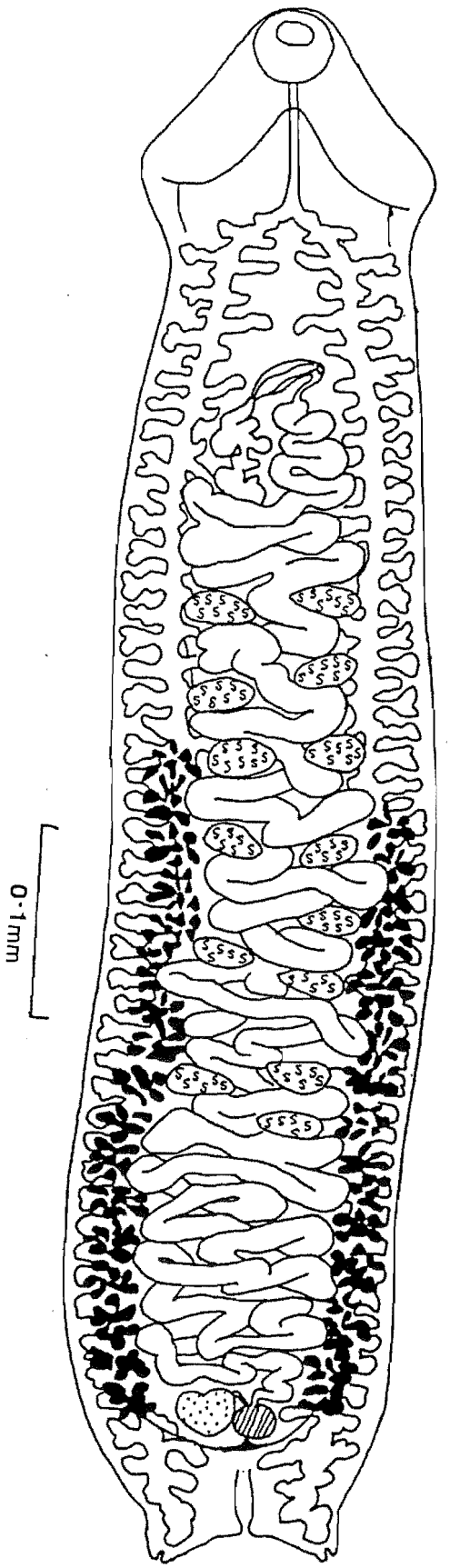
Charaxicephalus Looss, 1901 (Fig. 8.3)

Synonyms *Desmogonius* Stephens, 1911
Diaschistorchis Johnston, 1913
Charaxicephaloides Groschaft and Tenora, 1978

Emended diagnosis

Pronocephalidae. Body elongate or elliptical, canoe-shaped. Posterior papillate processes often present, either dome-shaped and setose, or conical and invaginated. Ventral glands absent. Head collar, when present, consisting of dorsal and ventral ridges; the latter may or may not be incised. Oral sucker terminal or sub-terminal. Caeca diverticulate or with crenated margins, extending to posterior of body. Excretory vesicle

Figure 8.3 *Charaxicephalus robustus* Looss, 1901 (after Yamaguti, 1972)



Y-shaped. Excretory vessels anastomosing or simple. Anterior region of excretory system unknown. Excretory pore dorsal. Testes follicular, rarely greater than twenty in number. Testicular follicles intercaecal, caecal or extracaecal, either in two parallel lateral fields or converging mediad at posterior extremity; completely preovarian, lateral to ovary or completely post-ovarian. Slender ejaculatory duct present or absent, cirrus sac enclosing prostate and sometimes terminal portion of seminal vesicle. Male and female genital pores separate or opening into a genital atrium; genital opening(s) below caecal bifurcation, intercaecal, caecal or extracaecal. Vitellaria in linear fields sometimes extending pre-equatorially. Ovary median or sub-median, above or level with Mehlis' gland. Uterine coils intercaecal, progressing anteriorly in transverse loops. Metraterm slender. Eggs with or without filaments. Parasites of aquatic turtles.

Type species: *Charaxicephalus robustus* Looss, 1901

Discussion

The distinguishing feature of *Charaxicephalus* is the presence of follicular testes. Amongst the species of the group, the arrangement of these follicles is variable, and has been used previously as diagnostic features to distinguish between sub-families and families. For instance, Price (1931) placed *Desmogonius* and *Charaxicephalus* in the Charaxicephalinae but excluded *Diaschistorchis* from the sub-family because the testicular follicles of the latter were lateral, to or below the ovary. However, according to the phylogenetic tree given in Fig. 6.11, such an arrangement results in a paraphyletic assemblage of member species, and nothing is gained by making the distinction.

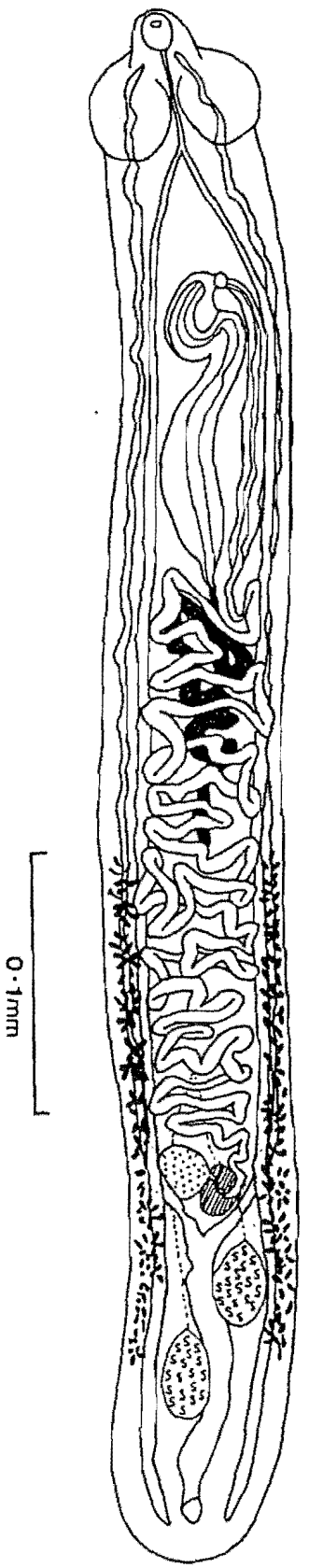
Pronocephalus Looss, 1899 (Fig. 8.4)

Synonyms. Opisthoporus Fukui, 1929
Teloporia Fukui, 1933

Emended diagnosis

Pronocephalidae. Body broadened in uterine zone, and sometimes attenuated at posterior extremity to form tail-like process. Head collar with dorsal ridge; ventral ridge complete or deeply incised. Caeca with smooth or crenated margins, extending to posterior of body. Excretory vesicle Y-shaped or trifurcated with one vessel extending into tail-like process. Excretory vessels simple, either uniting anteriorly, or

Figure 8.4 *Pronocephalus obliquus* Looss, 1899 (after Yamaguti, 1972).



terminating blindly. Excretory pore dorsal. Testes intercaecal and post-ovarian, either oblique or in tandem. Seminal vesicle long, tubular, strongly winding. Cirrus sac enclosing muscular ejaculatory duct, prostate, and sometimes part of seminal vesicle. Vitellaria in two linear and lateral fields beginning either pre- or post-equatorially. Ovary sub-median, anterior to or ventral to Mehlis' gland. Uterine coils overlapping caeca. Metraterm muscular. Genital opening common, intercaecal, median or sub-median. Eggs with polar filaments. Parasites of aquatic turtles.

Type species: *Pronocephalus obliquus* Looss, 1901; syn. *P. trigonocephalus* (Rud.) Looss, 1899

Discussion

The monotypic genus *Teloporia* is subsumed under *Pronocephalus* because of the placement of its testes (intercaecal) and the fact that its excretory vessels do not unite anteriorly. *P. aspidonectes* Fukui, 1929 (syn. *T. aspidonectes*) shares both features with *P. obliquus*. In *P. mehrai* Chattopadhyaya, 1972, however, the excretory vessels unite anterior to the caecal bifurcation.

P. aspidonectes is an interesting species (Fig. 8.5). The tail-like process at the posterior end of the body is unique, as is the trifurcation of the excretory vesicle into three vessels, two directed anteriorly, and one leading into the "tail". From Stunkard's (1930) diagram of an immature specimen of *P. aspidonectes* it appears that the "tail" appears as the animal matures, and that the "caudal" excretory vessel develops synchronously with the "tail". The immature animal therefore strongly resembles other pronocephalid species, and it is only the mature adult that is morphologically unique.

Cetiosaccus Gilbert, 1938 (Fig. 8.6)

Synonym. Metacetabulum Freitas and Lent, 1938

Emended diagnosis

Pronocephalidae. Body vermiform, longer than 5 mm. Dorsal ridge weakly developed or absent, ventral ridge incised or absent. Oral sucker terminal. Caeca simple, closely associated with excretory vessels, and terminating at the anterior margin of excretory bladder. Excretory bladder present, voluminous (approximately one-quarter body length), with concertina-like folds; may be eversible. Paired excretory vessels distinct, bifurcating at level of excretory bladder, diameter of posterior

Figure 8.5 *Pronocephalus aspidonectes* (Fukui, 1929) syn. *Teloporia aspidonectes* (after Stunkard, 1930).

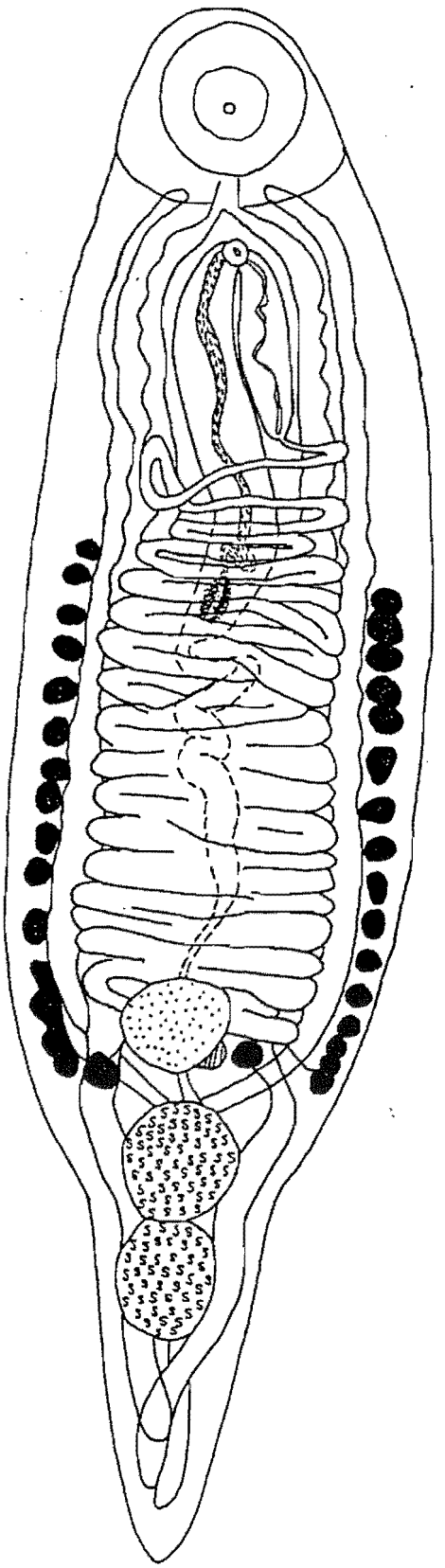
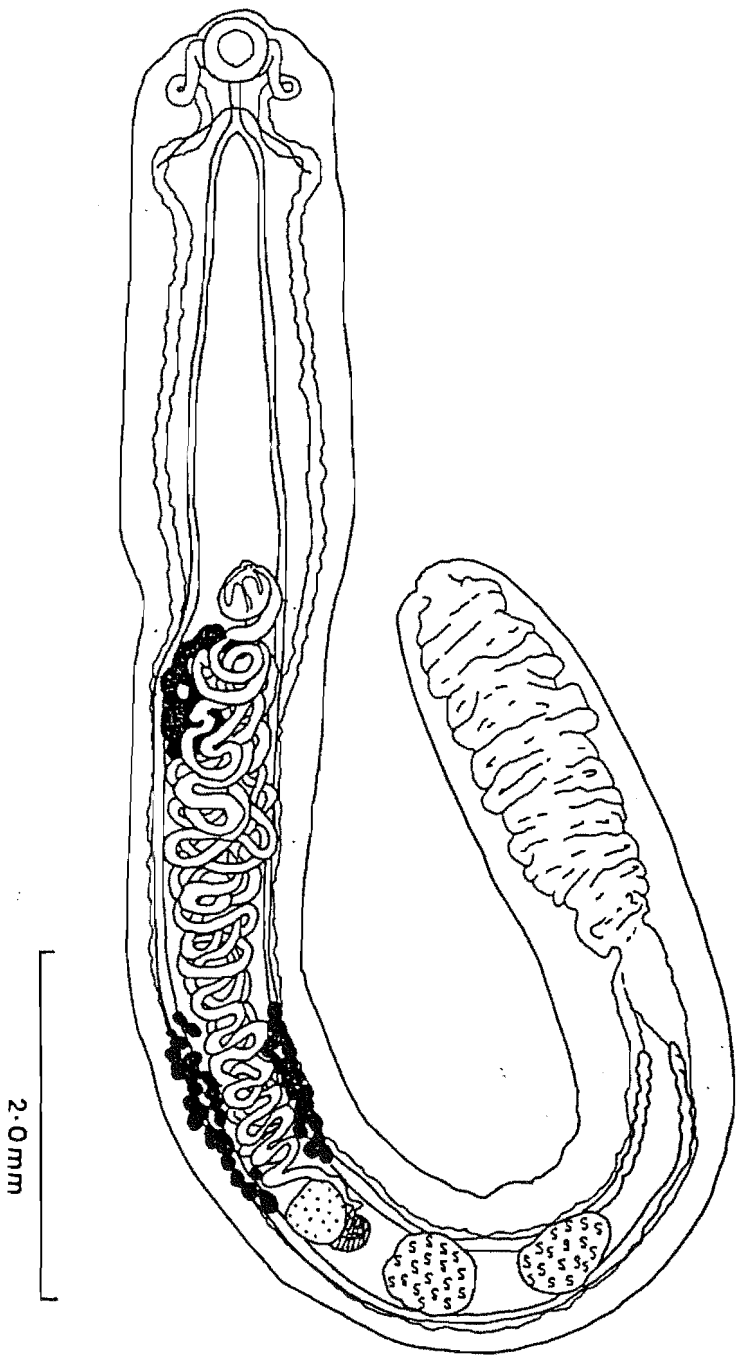


Figure 8.6 *Cetiosaccus galapagensis* Gilbert, 1938 (after Gilbert, 1938).



region of one vessel large than the other; meeting anteriorly simply or in an anastomosing network. Excretory pore terminal. Testes whole with smooth margins, intercaecal and arranged in tandem; postovarian. Long or short cirrus sac enclosing ejaculatory duct and papillate cirrus; sometimes enclosing prostate and terminal portion of seminal vesicle. Vitellaria in two short lateral fields in third quarter of body. Ovary median, above Mehlis' gland. Uterine coils disorderly or in transverse loops extending to pre-equatorial region, overlapping caeca. Metraterm muscular. Genital openings separate or common, intercaecal and sub-median. Eggs sometimes possessing a thick case or "shell", in which case polar filaments are absent. Parasites of marine turtles and iguanids.

Type species: *Cetiosaccus galapagensis* Gilbert, 1938

Discussion

Metacetabulum and *Cetiosaccus* are so similar that it was probably only an accident of timing that resulted in the establishment of both. Thus, both were erected in 1938, but the description of *Cetiosaccus* Gilbert was published in March, whereas the publication date of *Metacetabulum* Freitas and Lent was June.

Freitas and Lent (1938) erected the family Metacetabulidae to contain the genus *Metacetabulum*. Ruiz (1946) transferred the genus to the sub-family Choanoporinae Caballero, 1942 within the Pronocephalidae but Skrjabin (1955) reinstated the family Metacetabulidae to contain *Metacetabulum*. Later still, Yamaguti (1958) erected the sub-family Metacetabulinae within the Pronocephalidae for *Metacetabulum*. The primary source of controversy over the status of the genus stems from the vermiform appearance of the animal (unusual for pronocephalids) and the fact that the species of *Metacetabulum* lack a head collar. In addition, when Freitas and Lent first erected the genus, the modification of the excretory system to include a well-developed excretory bladder was not known in the Pronocephalidae. Freitas and Lent (1938) mistook the eversible bladder of *Metacetabulum* for an acetabulum and placed the family within the Paramphistomoidea. However, the structure of the reproductive organs and the close similarity of *Metacetabulum* to *Cetiosaccus* affirms the place of the former within the Pronocephalidae.

A major difference separating *C. galapagensis* from other members of the genus (and one which has been used previously to distinguish between *Cetiosaccus* and *Metacetabulum*), is the presence or absence of an eversible

bladdder. In *C. galapagensis* this feature is absent, whereas in other species of the genus it is present. However, in all other respects the structure of the excretory system is very similar.

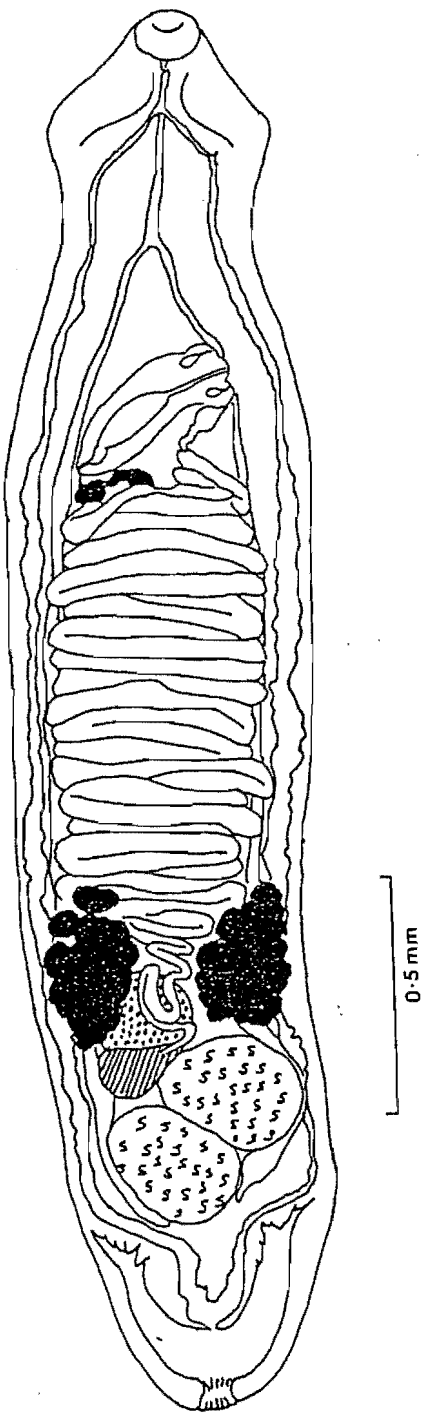
Another interesting feature noted by Gilbert (1938) is the difference in the diameters of the posterior sections of both excretory vessels of *C. galapagensis.* The illustration provided by Freitas and Lent (1938) indicates that this condition is also found in *C. invaginatus* (= *M. invaginatum* Freitas and Lent, 1938). My examination of the type specimen of *C. karachiense* (= *M. karachiense* Bilqees, 1974) also revealed this difference, but to a lesser degree. Interestingly, the diagram of a specimen of *C. invaginatus* with bladder everted in Freitas and Lent (1938) shows both limbs with the same diameter. Bilqees' (1974) type specimen also has an everted bladder. If there is a correlation between the asymmetry of the excretory vessels and a eversible bladder, then it is possible that *C. galapagensis* may also possess such a structure.

Macravestibulum Mackim, 1930 (Fig. 8.7)

Diagnosis (modified after Damian, 1961)

Pronocephalidae. Small trematodes, body elongate, canoe-shaped. Posterior extremity truncate or rounded. Dorsal cephalic ridge present, ventral ridge incised. Oral sucker sub-terminal. Caeca with smooth margins terminating posterior to testes and anterior to excretory bladder; enclosing testes, but converging mediad. Vestibular cavity present, bifurcated, eversible, opening to exterior by a large transverse slit. Excretory bladder well-developed but not voluminous, communicating with vestibular cavity by a median pore; bifurcated to give rise to two simple excretory vessels which unite above caecal bifurcation. Testes with smooth margins, intercaecal, oblique. Cirrus sac enclosing muscular ejaculatory duct, prostate, and part of seminal vesicle; also encloses two (possibly more) accessory vesicles and ducts; ducts opening into ejaculatory duct or on distal surface of inverted cirrus; ducts opening separately from ejaculatory duct in everted cirrus. Cirrus short and eversible. Vitellaria in two lateral compact fields. Ovary pre-testicular, sub-median, anterior to Mehlis' gland. Uterus in transverse coils extending pre-equatorially, sometimes overlapping caeca. Metraterm slender. Male and female terminal genitalia opening into a common atrium which is sub-median

Figure 8.7 *Macravestibulum kraatzi* Damian, 1961 (after Damian, 1961).



and intercaecal. Eggs unifilamentous at both ends. Parasites of North American freshwater turtles.

Type species: *Macravestibulum obtusicaudatum* Mackim, 1930

Discussion

Of all the genera, the classification of *Macravestibulum* is perhaps least controversial. However, the affinities of the group with other pronocephalid genera do highlight certain interesting points. These are discussed below.

Neopronocephalus Mehra, 1932 (Fig.8.8)

Diagnosis (modified after Yamaguti, 1972)

Pronocephalidae. Body elliptical, truncated at posterior end. Dorsal cephalic ridge and incised ventral ridge present. Oesophagus long, Caeca simple, rarely with crenated margins, terminating before fourth quarter of body. Vestibular cavity present, although it is more apparent in immature forms; opening terminal. Excretory bladder absent, excretory vessels bifurcating from short stalk above vestibule; uniting anteriorly or terminating blindly. Testes usually with smooth margins, rarely lobed, pre-ovarian and pre-vitelline, extracaecal or ventral to caeca. Cirrus sac muscular containing ejaculatory duct and prostate. Ovary sub-median, anterior to Mehlis' gland. Uterus first coiled behind ovary , passing between ovary and left testis and then between two testes. Metraterm muscular. Eggs with polar filaments. Parasites of Asian freshwater turtles.

Type species: *Neopronocephalus triangularis* Mehra, 1932, syn. *N. gangeticus* Mehra, 1932, *N. rotundus* Chatterji, 1936

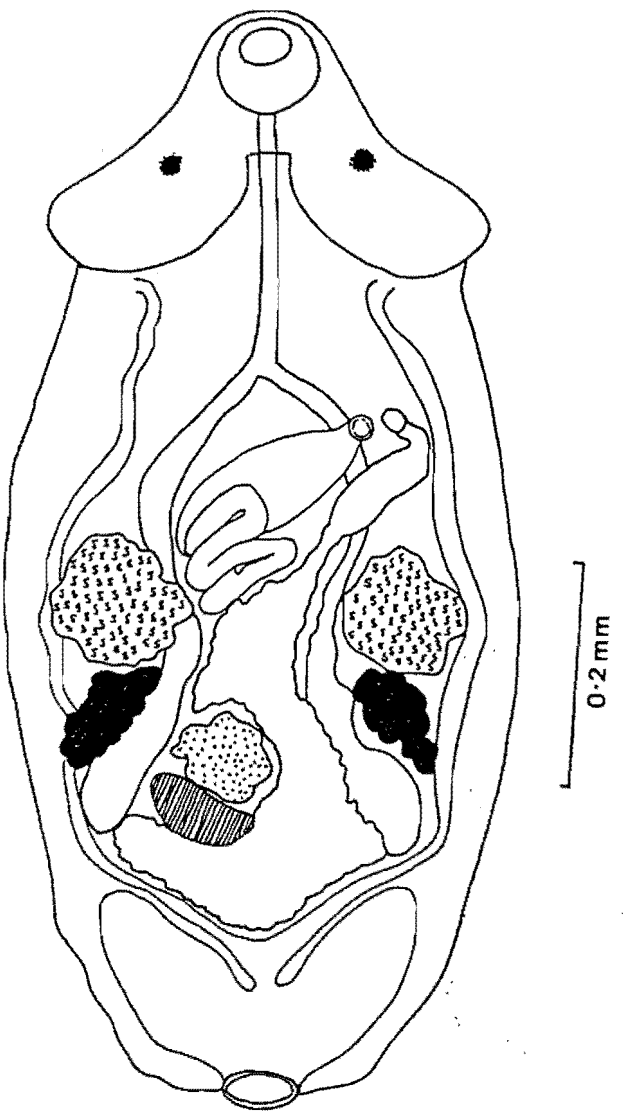
Discussion

In the diagnosis of *Neopronocephalus* given here, it is noted that a vestibular cavity (very much like that found in *Macravestibulum*) is present. This has not been noted before, and requires elaboration.

The following is Horsfall's (1930) description of the excretory system of *Cercaria infracaudata* Horsfall, 1930 (Fig. 8.9a):

"A large tube containing excretory granules forms a complete circuit along either side of the body from the region of the median eye spot to the excretory bladder and opens into the bladder in the anterior median line.

Figure 8.8 *Neopronocephalus triangularis* Mehra, 1932 (after Saxena, 1977).



> The excretory bladder is contractile and is constantly changing shape. It is a U-shaped structure, the length of the arms of the U depending upon its state of expansion."

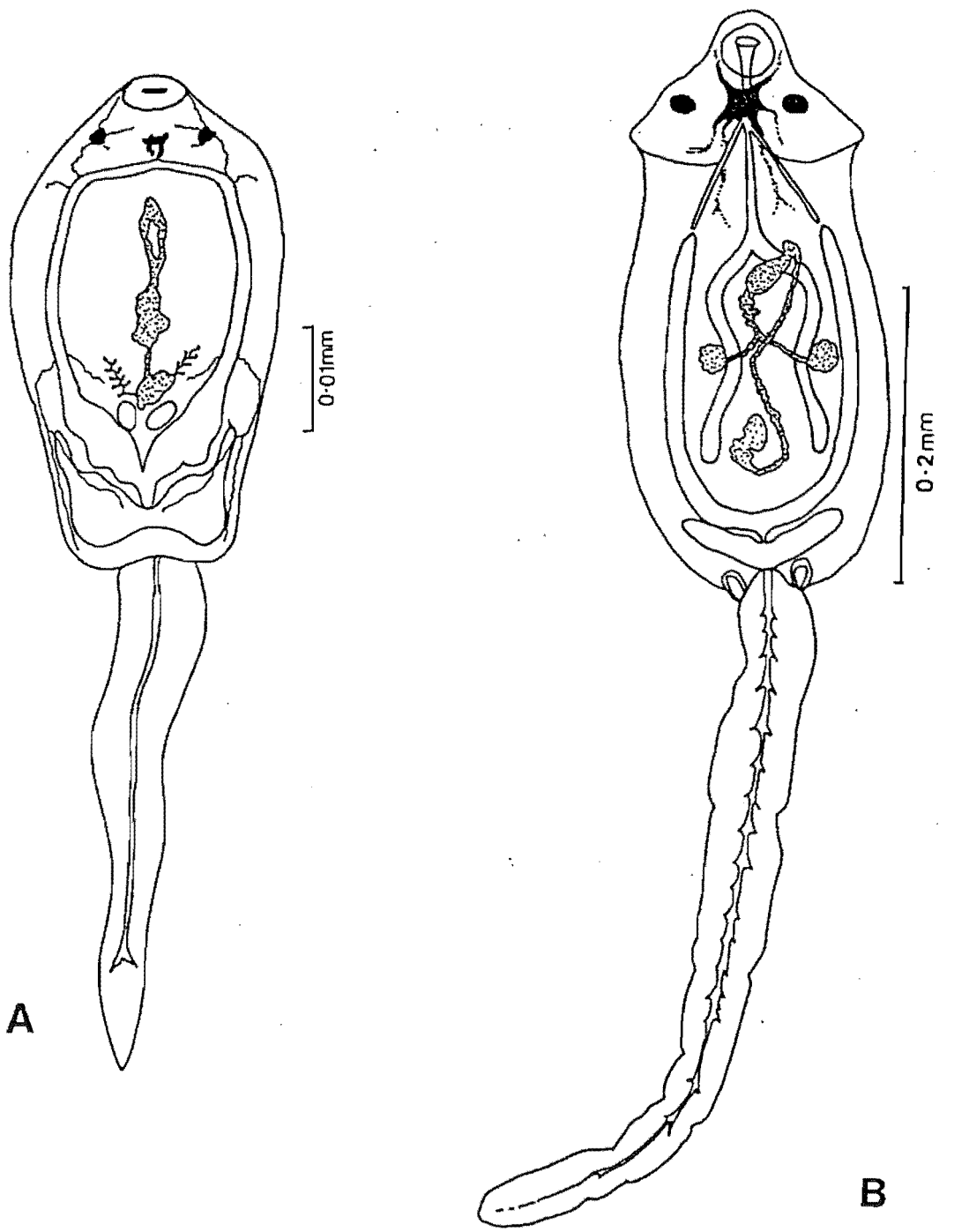
Compare this with the description of the excretory system of *Cercaria Neopronocephalus indicus* Thapar, 1968 (Fig. 8.9b):

> "The excretory bladder is large and more or less V-shaped with the two arms of the 'V' much expanded enclosing a wide obtuse angle. Immediately in contact with its anterior wall and passing forwards on either side of its lateral margins are the main excretory vessels filled with numerous rounded refractile excretory granules which communicate with the excretory bladder by a small duct opening into the anterior margin in the middle. Anteriorly the main excretory vessels could be traced as far as the level of the intestinal bifurcation, beyond which they are not clear, though in some specimens two faint ducts were seen passing forwards anteriorly, one from either side up to the median pigmented concentration."
>
> (Thapar, 1968)

The descriptions overlap significantly, but *C. infracaudata* it thought to be the juvenile of *Macravestibulum obtusicaudatum* whereas *C. Neopronocephalus indicus* is the juvenile of *N. indicus*. The adults of *M. obtusicaudatum* possess a vestibular cavity which is derived from the excretory bladder of the cercaria (Kuntz, 1951). Note that adults of *N. triangularis* have been described as possessing a thickwalled, V-shaped excretory bladder (Agarwal and Premvati, 1977). Certainly, this description can be applied equally to the vestibular cavity of *Macravestibulum* spp. and the "excretory bladder" of the adults of *N. triangularis* as illustrated by Agarwal and Premvati (1977) is remarkably similar to that of members of *Macravestibulum* (Fig. 6.7a).

Why is it that attention has not been drawn to the similarity between the vestibular cavity of *Macravestibulum* and the excretory bladder of *Neopronocephalus*? One possible reason is the state of contraction of the specimens and the cavity. Brooks and Palmieri (1979) in their description of *N. orientalis* Brooks and Palmieri, 1979 offer another clue. They say that the "excretory system [is] composed of Y-shaped excretory vesicle bifurcating

Figure 8.9 (A) *Cercaria infracaudata* Horsfall, 1930. Horsfall (1930) demonstrated that this is the cercarial form of *Macravestibulum obtusicaudatum* Mackim, 1930. (B) *Cercaria Neopronocephalus indicus* Thapar, 1968. This is the cercarial form of *Neopronocephalus triangularis* Mehra, 1932.



immediately posterior to posteriormost uterine extent... Portion of body containing excretory pore enclosed in velumlike posterior portion of body." This is the first reference to a "velumlike posterior portion of body", and if it is common amongst *Neopronocephalus* species, may obscure the vestibular cavity.

As early as 1946, Ruiz noted the close affinity of the genera *Neopronocephalus*, *Macravestibulum* and *Cetiosaccus*. The phylogenetic analysis given here supports that view. Other authors (e.g. Damian, 1961; Groschaft and Tenora, 1981) have argued that the genera should be placed in the same sub-family. Certainly the phylogenetic hypothesis presented here supports the close affinity of the genera.

Comments on the status of *Ruicephalus* and *Choanoporus*

Ruicephalus Skrjabin, 1955, contains the single species *R. minutus* (Ruiz, 1946) Skrjabin, 1955. Ruiz originally placed the species in *Pronocephalus* because its testes were almost completely intercaecal. However, *R. minutus* is unique in the structure of its head collar (which consists of a well developed flange-like ridge incised both ventrally and dorsally), the presence of what appears to be a small pharynx or pharyngeal bulb, and a modification to the posterior portion of the body which has not been adequately described. The structure of the excretory system is unknown, and the position of the ovary (pre-vitelline) is not seen in other pronocephalid species. In fact, the genus seems to be included within the Pronocephalidae only because it possesses some form of head collar. I believe that this alone is not sufficient to warrant membership in the family. Because of the major dissimilarity between *R. minutus* and other pronocephalids, I was unable to include it in the analysis.

Choanoporus rovirosai Caballero, 1942, the single species of *Choanoporus* Caballero, 1942, however, is a reasonably well described species. It is vermiform, and the position of its reproductive organs seem to be similar to those of *Cetiosaccus* and *Macravestibulum*. Also, the excretory system seems to be extensively modified. However, the precise nature of this modification is not known. It appears that the excretory bladder (?) is bifurcated, suggesting that it may be similar to that of *Neopronocephalus* and *Macravestibulum*. However, in the drawing of *C. rovirosai* given by Caballero (1942), the excretory bladder appears very much like that of *Cetiosaccus*. Almost certainly, *Choanoporus* is closely related to the generic group comprising *Cetiosaccus*, *Macravestibulum*, and *Neopronocephalus*. However, the precise nature of this relationship will not be known until the excretory system of *Choanoporus* is studied in greater detail.

SUMMARY

A revision of the generic classification of the Pronocephalidae Looss 1902 is presented. The family consists of seven genera:

1. *Notocotyloides*, distinguished by the possession of a head collar, absence of ventral glands, and ovary posterior to Mehlis' gland;

2. *Pleurogonius*, distinguished by the ovary anterior to Mehlis' gland, whole testes lying mainly in the post-ovarian region of the body, and largely extracaecal;

3. *Charaxicephalus*, distinguished by the possession of follicular testes;

4. *Pronocephalus*, distinguished by the possession of a head collar, intracaecal testes, and simple excretory system;

5. *Cetiosaccus*, distinguished by a vermiform body and the presence of a voluminous bladder;

6. *Macravestibulum*, distinguished by the presence of a vestibular cavity and intercaecal testes; and

7. *Neopronocephalus*, distinguished by the presence of the vestibular cavity but with pre-ovarian testes.

GENERAL CONCLUSIONS

GENERAL CONCLUSION

Phylogenetic taxonomy: cladistics through the looking glass

> "Then she began looking about, and noticed that what could be seen from the old room was quite common and uninteresting, but that all the rest was as different as possible. For instance, the pictures on the wall next the fire seemed to be all alive, and the very clock on the chimney-piece (you know you can only see the back of it in the Looking-glass) had got the face of a little old man, and grinned at her.
>
> *Alice through the Looking-Glass*

When Alice stepped through the looking glass, she saw a world which bore a passing resemblance to her own, but did not quite match up. The orderliness which she had come to expect, and which she was prepared to deal with, was no more. Instead, she was faced with a plethora of new experiences, in which the logic and rationality of the world she had just left was often inapplicable.

The experiences of Alice and those of contemporary theoretical systematists are strikingly parallel. Just like Alice, theoretical systematists often develop their methods in a world of perfect (or almost perfect) data. In such a world, controversies in phylogenetic reconstruction centre on the appropriateness of techniques, and only rarely on discussions about the effects of data that violate the assumptions of these techniques to varying extents. When these systematists "step through the looking glass" into the real world of applied taxonomy, it is often apparent that the suite of techniques they have developed are suited for "best-case" taxonomic data. How do these systematists deal with the kind of data which practising taxonomists face daily? In conversations with a few theoretical systematists, the response has often been "accumulate more data" or "refine the data" or "don't analyse the data". In other words, there is a feeling that such data is at best, preliminary, and at worst, useless. Often however, it is the *only* data that is available, and in all probability, the only data that *will be* available for a long while.

In this thesis, I have taken the attitude that an hypothesis of relationship is better than no hypothesis. It is essential to understand that an hypothesis represents more than a statement of one's belief or views; rather, it serves as a means to *direct* future research:

An hypothesis offers us a framework within which a directed protocol for research can be developed.

And this is how science progresses: in attempting to disprove hypotheses, research is implicitly or explicitly integrated, new facts are accumulated, and should such hypotheses be falsified, new hypotheses are erected. In such a scheme of things, it is not enough to say that data is not good enough to develop a "good" hypothesis, because *any* scientific hypothesis which generates and focusses research is good.

The methods I have used are founded on the principles of phylogenetic systematics. These principles include the following:

1. Classifications should reflect phylogeny, i.e., closely related groups should share a common ancestor, not shared by other groups.

2. In order for the phylogeny of a group to be reconstructed, enough characters must be available and the evolutionary polarity of their states must be known. Shared derived states suggest closeness of relationships or morphogenetic tendency. It is also desirable that the rates of character change be known.

3. Taxonomic categories should be allocated so as to minimise redundancies.

However, I believe that the principles I have discussed and applied in this thesis, form the basis of a sub-discipline which I will call *Phylogenetic Taxonomy*. Briefly stated, phylogenetic taxonomy is the application of the principles and methods of phylogenetic systematics subject to the constraints of applied taxonomy. These constraints include:

1. Having to work within the framework of the International Codes of Nomenclature. This means, among other things, having to accept an upper limit to the number of categories formally available.

2. The unavailability of live specimens or specimens in sufficient numbers to assess intra-taxon variability of characters, and the absence of type specimens.

3. The inconsistencies of published descriptions.

4. The fact that classifications are not an end in itself, but serve as a database for comparative biologists and biological resource managers. As a result, stability of classifications is a desirable property.

On the basis of these constraints, a preliminary set of principles of phylogenetic taxonomy can be tabled. These are:

1. Classifications should reflect phylogeny (in the sense given above) *unless* groups are erected on the basis of "questionable" characters, or characters with low weights. In such cases, paraphyletic classification is

acceptable, if the monophyly of the next higher category is preserved.

2. Some form of character weighting is necessary, either on the basis of *a priori* information, or on the basis of a successive approximations approach. In the latter case, trees should be given a high or low confidence value depending on whether the characters which are weighted highly are also those which are considered to be "well-defined".

3. Character weights should be taken to be as hypotheses of evolutionary stability, and observational tests of these hypotheses should be undertaken. In addition, hypotheses of parallelisms should be tested by recourse to ontogenetic or structural data.

In the fullness of time, I hope to be able to develop these principles more lucidly. However, in applying these principles to the classification of the Pronocephalidae, I believe that I have illustrated by example the main tenets of phylogenetic taxonomy.

ACKNOWLEDGEMENTS

I have not worked in isolation. There have been many people who have helped me. Some of them do not know it, but often a comment uttered lightly or a casual query would sow the seeds of an idea, and sometime later it would crystallise in my mind. As a result, I have a great many people to thank. First and foremost, Dr. Mike Winterbourn, Mr. Peter Johns, and Dr. David Blair, my long suffering supervisors, have my utmost appreciation. They let me work the way I like to work, and if this thesis shows some glimmer of originality, it is because they did not restrain me from wandering onto paths unknown. Dr. Dan Brooks, although he does not know this, was responsible for igniting my enthusiasm in systematics, and I am grateful to him for this. Dr. Robert Jackson has always been forthcoming in his encouragment, even though there was a time when I was not too nice to his spiders. Dr. Bob Murphy, the former Principal of Rochester and Rutherford Halls, convinced me to continue my work when I reached that dark period which all graduate students go through (or so I am told).

There have been many others I have had discussions with: Dr. Steve Barker, Dr. Phil Garnock-Jones, Dr. Russell Gray, Mr. Richard Holdaway, Dr. Ian Henderson, Mr. Roderick Page, Prof. John Pearson, Dr. David Penny, and Dr. Murray Smith. These people have offered comments and suggestions, which have contributed to the ideas expressed in this thesis. I thank all of them. Special thanks must also go to the secretaries of the Zoology Department, University of Canterbury, and in particular, Tracy Robinson, who helped me with the arduous task of printing out this thesis. Terry Williams, the photographic technician, whom I harassed whenever I needed slides for conferences, also has my gratitude. Jan McKenzie's help with the microscope work (both EM and light microscopy) was invaluable and is greatly appreciated. Karen Claxton did many of the drawings in this thesis, and this certainly helped when I had the "shakes". Yvette Rodrigo (my sister) devoted many long afternoons to compiling Table 6.4.

Finally, my colleagues in the Zoology Department, University of Canterbury, kept me sane; my partner and friend, Karen Claxton kept me happy; and my

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APPENDIX

The derivation of the Optimal Likelihood Index

APPENDIX

The likelihood, $L_{.T}$, of phylogenetic tree and a particular set of evolutionary changes, T, is the product of the likelihood of p characters:

$$L_{.T} = \prod_{i=1}^{p} L_i \qquad (1)$$

where L_{iT} is the likelihood of the ith character.

For each character, we assume that the transformation probabilities are small, relative to the probability of retaining the same state.

The character likelihood function for character i, on tree T is calculated as:

$$L_{iT} = \prod_{j=1}^{t} P_{ij} \qquad (2)$$

where j is a branch-labelling variable;

and t is the number of OTUs.

Depending on the character state assignment on branch j, P_{ij} is the probability of character i changing or retaining the same state. Taking logs on both sides we get

$$\ln L_{iT} = \sum_{j} \ln P_{ij} \qquad (3)$$

If character i is hypothesised as having c_{iT} changes, for the tree T, then

$$\ln L_{iT} = c_{iT}.\ln P_{iC} + (2t-2-c_{ik}).\ln P_{iNC} \qquad (4)$$

where P_{iC} and P_{iNC} are the probabilities associated with one character state change, and no character state changes, of character i, respectively.

This gives us

$$\ln L_{iT} = c_{iT}.\ln P_{iC} + (2t-2).\ln P_{iNC} - c_{iT}.\ln P_{iNC}$$

$$= c_{iT}(\ln P_{iC} - \ln P_{iNC}) + (2t-2).\ln P_{iNC} \qquad (5)$$

If the probabilities of change are sufficiently small, we

can estimate the probability of a single change, for character i, as:

$$P_{iC} = e^{-r_i} . r_i \qquad (6a)$$

and that of no change as:

$$P_{iNC} = e^{-r_i} \qquad (6b)$$

Substituting (6a),(6b), and into (5), we get

$$\ln L_{iT} = c_{iT} \ln r_i - (2t-2)\, r_i \qquad (7)$$

When r_i is unknown, $\ln L_{iT}$ may be calculated by integrating with respect to r_i, over an appropriate range. However, when we have no knowledge of what this range might be, it is possible to estimate it using the different number of character changes for each tree.

If, for the kth tree, the number of character changes for the ith character is used to estimate r_i as follows:

$$r_{ik} = c_{ik}/(2t-2), \qquad (8)$$

then the range of possible values of r_i include all such estimates. Since there are a finite number of estimates, $\ln L_{iT}$ may be derived by summing over all r_{ik}.

$$\ln L_{iT} = c_{iT} \left(\sum_k \ln r_{ik}\right) - (2t-2)\sum_k r_{ik}$$

$$= c_{iT} \left\{\ln \prod_k [c_{ik}/(2t-2)]\right\} - \sum_k c_{ik} \qquad (9)$$

where E is the total number of trees.

The log-likelihood over all characters is

$$\ln L_{.T} = \underbrace{\sum_i c_{iT} \ln \prod_k c_{ik}}_{\text{Term 1}} - \underbrace{E \ln(2t-2) \sum_i c_{iT}}_{\text{Term 2}} - \underbrace{\sum_i \sum_k c_{ik}}_{\text{Term 3}} \qquad (10)$$

Terms 2 and 3 are constants. Maximising the likelihood function, therefore, involves finding a tree, T, such that

$$L'_{.T} = c_{iT} \ln \prod c_{ik}, \qquad (11)$$

is maximised.

Multiplying throughout by $1/\underline{p}$ where $\underline{p}$ is the number of characters, we get

$$\underline{L'}_{.T} = \sum \underline{c}_{iT} \ln\left(\prod \underline{c}_{ik}\right)^{1/p} \qquad (12)$$

or

$$\underline{L'}_{.T} = \sum \underline{c}_{iT} \ln\left(\overline{\underline{c}_{ik}}\right)$$

where $\overline{\underline{c}_{ik}}$ is the geometric mean of the number of changes for character $\underline{i}$.

REFERENCES

REFERENCES

Abbott, L.A., Bisby, F.A. and Rogers, D.J. (1985) Taxonomic Analysis in Biology. Columbia University Press. New York.

Agarwal, M. and Premvati, G. (1977) Juveniles and adults of *Neopronocephalus triangularis* Mehra, 1932 (Trematoda: Pronocephalidae) with remarks on species of the genus. Ind. J. Parasitol. 1: 147-149.

Archie, J.W. and Felsenstein, J. (1989, in press) The number of evolutionary steps on random and minimum length trees for random evolutionary data. Theoret. Pop. Biol.

Barry, D. and Hartigan, J.A. (1987) Statistical analysis of hominoid molecular evolution. Statistical Science 42:191-210.

Beverly-Burton, M. and Logan, V.H. (1972) The ventral papillae of notocotylid trematodes. J. Parasitol. 62: 148-151.

Bhatnagar, A.K. and Gupta, A.N. (1981) New morphogenic features in the female genital complex of *Neopronocephalus triangularis* (digenetic trematode). Folia Parasitologica 28:343-347.

Bilqees, F.M. (1974) Two species of trematoda of *Chelonia mydas* from Karachi coast, Pakistan. Acta Parasitol. Polon. 22: 295-303.

Blackwelder, R.E. (1967) Taxonomy. John Wiley and Sons, New York.

Boehner, P. (1957) Ockham - Philosophical Writings. Bobbs-Merrill, Indianapolis.

Brooks, D.R. and Palmieri, J.R. (1978) Pronocephalid trematodes from a Malaysian turtle including a new species of *Renigonius* Mehra, 1939. Proc. Helm. Soc. Wash. 45: 34-36.

Brooks, D.R. and Palmieri, J.R. (1979) *Neopronocephalus orientalis* sp. n. (Digenea:Pronocephalidae) and *Spirhapalum elongatum* Rohde, Lee and Lim, 1978 (Digenea:Spirochiidae) from *Cuora amboinensis* (Daudin) in Malaysia. Proc. Helm. Soc. Wash. 46: 55-57.

Brooks, D.R., Caira, J.N., Platt, T.R. and Pritchard, M.H. (1984) Principles and Methods of Phylogenetic Systematics: a Cladistics workbook. University of Kansas, Museum of Natural History Special Publication No. 12.

Brooks, D.R., O'Grady, R.T. and Wiley, E.O. (1987) A measure of the information content of phylogenetic trees, and its use as an optimality criterion. Syst. Zool. 35: 571-581.

Brown, W.M., Prager, E.M., Wang, A., and Wilson, A.C. (1987). Mitochondrial DNA sequences of primates. Tempo and mode of evolution. J. Mol. Evol. 18:225-239.

Bryant, H.N. (1989) An evaluation of cladistic and charcter analyses as hypothetico-deductive procedures, and the consequences for character weighting. Syst. Zool. 38: 214-227.

Caballero, C.E. (1942) Trematodos de las tortugas de Mexico II. Descripcion de un nuevo genero de la familia Pronocephalidae Looss, 1902 y descripcion de una nueva especie del genero *Octangioides* Price, 1937. Ann. Inst. Biol. 13: 81-90.

Caballero, C.E. (1954) Helminths de la Republica de Panama X. Algunos Trematodos de *Chelone mydas* (L) tortuga marina comestible del Oceano Pacifico del Norte. An. Esc. Nac. Cien. Biol. 8: 31-58.

Camin, J.H. and Sokal, R.R. (1965) A method for deducing branching sequences in phylogeny. Evolution 19: 311-326.

Cavender, J.A. (1981) Tests of phylogenetic hypotheses under generalised models. Math. Biosci. 54: 217-229.

Chattopadhyaya, D.R. (1972) Studies on the trematode parasites of reptiles found in India. Contribution to our knowledge of the family Pronocephalidae Looss, 1902.. Rivista di Parasitol. 33: 99-124.

Coil, W.H. and Reid, W.A. (1975) *Desmogonius desmogonius* Stephens, 1911 (Pronocephalidae) a redescription with observations on egg filament formation. Z. Parasit. 25: 506-509.

Cracraft, J. (1974) Phylogenetic models and classification. Syst. Zool. 23: 71-90.

Cracraft, J. (1983) The significance of phylogenetic classifications for systematic and evolutionary biology. in Numerical Taxonomy (ed. Felsenstein, J.). Springer- Verlag, Berlin.

Cronquist, A. (1969) On the relationship between taxonomy and evolution. Taxon 18:177-187.

Crusz, H. and Fernand, V.S. (1954) The trematode parasites of the dugong with description of the new monostomes and histological changes in the host. J. Parasitol. 40: 499-507.

Damian, R.T. (1961) *Macravestibulum kraatzi* n. sp. (Trematoda: Pronocephalidae), with revision of the genus *Macravestibulum* Mackim, 1930. J. Parasitol. 47: 503-507

Danks, H.V. (1988) Systematics in support of entomology. Ann. Rev. Entomol. 33: 271-296.

Darwin, C. (1859) The Origin of the Species. John Murray, London.

Doyen, J.T. and Slobodchikoff, C.N. (1974) An operational approach to species classification. Syst. Zool. 23: 239-247.

Dummett, M. (1981) Frege: philosophy of language. Harvard University Press, Massachusetts

Dwivedi, M.P. (1977) Description of three new species of the genus *Neopronocephalus* Mehra, 1932 (Pronocephalidae: Trematoda). Rivista di Parasitol. 38: 319-327.

Estabrook, G.F., Strauch, J.G. and Fiala, K.L. (1977) An application of compatibility analysis to the Blackiths' data on orthopteroid insects. Syst. Zool. 26: 269-276.

Estabrook, G.F. (1978) Cladistic methodology: a discussion of the theoretical basis for the induction of evolutionary history. Ann. Rev. Ecol. Syst. 3: 427-456.

Estabrook, G.F. (1984) Phylogenetic trees and character-state trees in Cladistics: perspectives in evolutionary reconstruction (eds. Duncan, T. and Stuessy, T.F.) Columbia University Press, New York.

Faith, D.P. (1983) Parsimony and paraphyly. in Numerical Taxonomy (ed. Felsenstein, J.). Springer- Verlag, Berlin.

Faith, D.P. (1983) Information content and most parsimonious trees. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Farris, J.S. (1969) A successive approximations approach to character weighting. Syst. Zool. 18: 374-385.

Farris, J.S. (1972) Estimating phylogenetic trees from distance matrices. Am. Nat. 106: 645-668.

Farris, J.S. (1973) A probability model for inferring evolutionary trees. Syst. Zool. 22: 250-256.

Farris, J.S. (1977) Parsimony under Dollo's Law. Syst. Zool. 26: 77-88.

Farris, J.S. (1979) The information content of the phylogenetic system. Syst. Zool. 28: 483-519.

Farris, J.S. (1980) The efficient diagnoses of the phylogenetic system. Syst. Zool. 29: 386-401.

Farris, J.S. (1983) The logical basis of phylogenetic systematics. in Advances in Cladistics, Vol 2: Proceedings of the Second Meeting of the Willi Hennig Society (eds. Platnick, N.I. and Funk, V.A.). Columbia University Press, New York.

Fauchald, K. (1974) Polychaete phylogeny: a problem in protostome evolution. Syst. Zool. 23: 493-506.

Felsenstein, J. (1973) Maximum likelihood and minimum steps methods for estimating evolutionary trees from data on discrete characters. Syst. Zool. 22: 240-249.

Felsenstein, J. (1978) Cases in which parsimony and compatibility methods will be positively misleading. Syst. Zool. 27: 401-410.

Felsenstein, J. (1979) Alternative methods of phylogenetic inference and their interrelationship. Syst. Zool. 28: 49-62.

Felsenstein, J. (1981) A likelihood approach to character weighting and what it tells us about parsimony and compatibility. Biol. J. Linn. Soc. 16: 183-196.

Felsenstein, J. (1982) Numerical methods for inferring evolutionary trees. Quart. Rev. Biol. 57: 379-404.

Felsenstein, J. (1983) Methods for inferring phylogenies: a statistical view. in Numerical Taxonomy (ed. Felsentein, J.). Springer-Verlag, Berlin

Felsenstein, J. (1983) Statistical inference of phylogenies. J. Roy. Statist. Soc. A 146: 246-272.

Felsenstein, J. (1984) The statistical approach to inferring evolutionary trees and what it tells us about parsimony and compatibility. in Cladistics: perspectives in evolutionary reconstruction (eds. Duncan, T. and Stuessy, T.F.) Columbia University Press New York.

Felsenstein, J. (1987) PHYLIP (Phylogenetic Inference Package) Ver. 3.0 Manual. University of Washington (distributed on floppy disks)

Felsenstein, J. (1988) Phylogenies from molecular sequence: inference and reliability. Ann. Rev. Genet. 22: 521-565.

Fiala, K.L. (1983) A simulation model for numerical taxonomic methods. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin

Fischthal, J.H. and Acholonu, A.D. (1976) Some digenetic trematodes from the Atlantic hawksbill turtle, *Eretmochelys imbricata imbricata* (L.) from Puerto Rico. Proc. Helm. Soc. Wash. 42: 174-185.

Funk, V.A. (1983) The value of natural classification. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Gaffney, E.S. (1979) An introduction to the logic of phylogeny reconstruction in Phylogenetic Analysis and Paleontology (eds. Cracraft, J. and Eldredge, N.) Columbia University Press, New York.

Gosliner, T.M. and Ghiselin, M.T. (1984) Parallel evolution in opisthobranch gastropods and its implications for phylogenetic methodology.. Syst. Zool. 33: 255-276.

Gould, S.J. (1985) The flamingo's smile. Penguin Books, England.

Groschaft, J. and Tenora, F. (1978) *Charaxicephaloides polyorchis* gen. n. sp. n. (Trematoda: Charaxicephalidae) from *Chelonia mydas mydas* (Testudinata) in Cuba. Vestn. Cs. spol. zool. 42: 108-111.

Gupta, S.P. (1962) On two new trematodes from the intestine of the marine turtle, *Chelone mydas* from the Carribean Sea. Ind. J. Helminth. 14: 71-76.

Harre, R. (1986) The Philosophies of Science. Second Edition. Oxford University Press. United Kingdom.

Hecht, M.K. and Edwards, J.L. (1976) The methodology of phylogenetic inference above the species level. in Major Patterns in Vertebrate Evolution (eds. Hecht, M.K., Goody, P.C., and Hecht, B. M.). Plenum Press, New York.

Hendy, M.D. and Penny, D. (MS) A framework for the quantitative study of evolutionary trees.

Hennig, W. (1950)Grundzuge einer Theorie der phylogenetischen Systematik. Deutscher Zentralverlag, Berlin. 1950.

Hennig, W. (1965) Phylogenetic systematics. Ann. Rev. Entom. 10: 97-116.

Hennig, W. (1966) Phylogenetic Systematics. Translated by Davis, D.D. and Zangerl, R. University of Illinois Press, Urbana, 1979.

Heywood, V.H. (1984) The current scene in plant taxonomy. in Current Concepts in Plant Taxonomy (eds. Heywood, V.H. and Moore, D.M.). Academic Press, U.K.

Hollings, E. and Stace, C.A. (1982) Morphological variation in Vicia sativa L. aggregate. Watsonia 12: 1-14.

Horsfall, M.W. (1935) Observations on the life history of *Macravestibulum obtusicaudatum* Mackim, 1930 (Trematoda: Pronocephalidae). Proc. Helm. Soc. Wash. 28: 111-114.

Hsu, D.Y.M. (1937) Life history and morphology of *Macravestibulum eversum* sp. nov. (Pronocephalidae, Trematoda). Trans. Am. Micr. Soc. 56: 478-504.

Hull, D. (1988) Science as a Process: an evolutionary account of the social and conceptual development of science. University of Chicago Press, Chicago.

Hunter, W.S. (1961) A new monostome *Pleurogonius malaclemys* n. sp. (Trematoda: Pronocephalidae) from Beauford, North Carolina. Proc. Helm. Soc. Wash. 28: 111-114.

Jahan, A. (1970) Studies on digenetic trematodes of vertebrates. On four trematode parasites of turtles. Ann. Parasit. Hum. Comp. 45: 793-803.

Jensen, R.J. (1983) A practical view of numerical taxonomy or should I be a pheneticist or a cladist? in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Katz, M. J. (1987) Is evolution random ? in Development as an Evolutionary Process. Alan R. Liss, Inc. USA.

Kiriakoff, S.G. (1959) Phylogenetic systematics versus typology. Syst. Zool. 8: 117-118.

Kluge, A.G. and Farris, J.S. (1969) Quantitative phyletics and the anurans. Syst. Zool. 18: 1-32.

Kluge, A. G. (1983) The relevance of parsimony in phylogenetic inference in Cladistics: perspectives on the reconstruction of evolutionary history (eds. Duncan, T and Stuessy, T. F.) Columbia University Press. New York.

Kobayashi, H. (1921) On some digenetic trematodes in Japan. Parasitology 12: 380-410.

Kuhn, T. (1970) The Structure of Scientific Revolutions. Second Edition. University of Chicago Press, Chicago.

Kuntz, R.E. (1951) Embryonic development of the excretory system in a psilostome cercaria, a gymnocephalous (fasciolid) cercaria and in three monostome cercaria. Trans. Am. Micr. Soc. 70: 95-118.

Lakatos, I (1965). The Methodology of Scientific Research Programmes. Cambridge University Press, U.K.

Lakshman Rao, S. (1975) Studies on the trematodes of turtles from India. Further contribution to our knowledge of the family Pronocephalidae Looss, 1902. Rivista di Parasitol. 36: 137-151.

Lambert, D.M., Michaux, B. and White, C.S. (1987) Are species self-defining?. Syst. Zool. 36: 196-205.

Lloyd, D.G. and Calder, V.L. (MS) Multi-residue gaps, a class of molecular characters with exceptional reliability for phylogenetic analysis.

Looss, A. (1899) Weitere Beitrage zur Kenntnis der Trematoden-Fauna Aegyptens, zugleich Versuch einer naturlichen Gliederung des Genus Distomum Retzius. Zool. Jb. Syst. Geogr. Biol. : 521-784.

Looss, A (1901) Notizen zur Helminthologie Egyptens. IV. Ueber Trematoden and Seeschildkroten der egyptischen Kusten.. Zbl. Bakt. Parasit. Infekt. 30: 555-569.

Looss, A., (1902) Ueber neue und bekannte Trematoden aus Seeschildkroten. Zool. Jb. 16: 411-894.

Lossee, J. (1980) A Historical Introduction to the Philosophy of Science. Second Edition. Oxford University Press, U.K.

Mahalanobis, P.C. (1936) On the generalised distance in statistics. Proc. Natl. Inst. Sci. India 2: 49-55.

Mayr, E. (1942) Systematics and the Origin of the Species. Columbia University Press, New York.

Mayr, E. (1969) Principles of Systematic Zoology. McGraw-Hill, New York.

Mayr, E. (1970) Populations, Species, and Evolution. Harvard University Press, Cambridge.

Mayr, E. (1982) The Growth of Biological Thought. Bellknap Press of Harvard University Press, Massachusetts.

McNeill, J. (1983) The future of numerical methods in plant systematics: a personal prospect. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Meacham, C.A. and Estabrook, G.F. (1985) Compatibility methods in systematics. Ann. Rev. Ecol. Syst. 16: 431-446.

Meacham, C.A. (1980) Phylogeny of the Berberidaceae with an evaluation of classifications. Syst. Bot. 5: 149-172.

Mehra, H.R. (1932) Classification de la famile Pronocephalidae Looss. Ann. Parasitol. 10: 323-329.

Mehra, H.R. (1932) Note sur le genre *Neopronocephalus* Mehra, 1932. Ann. Parasitol. 10: 544.

Mehra, H.R. (1932) Noveaux monostomes e la famile des Pronocephalidae des tortues d'eaue douce de l'Inde. Classification de cette famille. Ann. Parasitol. 10: 225-247.

Mehra, R.K. (1939) New monostomes of the family Pronocephallidae Looss, 1902. Proc. Nat. Acad. Sci. India 9: 99-130.

Mehrotra, V. and Gupta, N.K. (1977) On two species of the genus *Diaschistorchis* Johnston, 1913 (Trematoda: Pronocephalidae) from marine turtles in India. Rivista di Parasitol. 37: 279-286.

Mikevich, M.F. (1978) Taxonomic congruence. Syst. Zool. 27: 143-158.

Mitchison, N.A. (1980) Inheritance: the immunological challenge. Nature 288: 306.

Moody, S.M. and O'Nolan, P. (1987) An a priori character weighting method for cladistic analysis (Abstract). Am. Zool. 26: 108A.

Moss, W.M. (1983) Taxa, taxonomists, and taxonomy. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Neff, N.A. (1986) A rational basis for a priori character weighting. Syst. Zool. 35: 110-123.

Nelson, G. and Platnick, N.I. (1981) Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.

Newton-Smith, W.H. (1981) The Rationality of Science. Routledge and Kegan Paul, U.K.

Panchen, A.L. (1982) The use of parsimony in testing phylogenetic hypotheses. Zool. J. Linn. Soc. 74: 305-328.

Patterson, C. (1982) Morphological characters and homology. in Problems of Phylogenetic Reconstruction (eds. Joysey, K.A. and Friday, A. E.). Academic Press, London.

Penny, D. and Hendy, M.D. (1985) Testing methods of evolutionary tree construction. Cladistics 1: 266-272. Penny, D., Hendy, M.D. and Steel, M. (MS) Accuracy of tree reconstruction methods.

Phillips, R.B. (1984) Considerations in formalising a classification in Cladistics: perspectives on evolutionary reconstruction (eds. Duncan, T. and Stuessy, T.F.) Columbia University Press, New York.

Popper, K.R. (1934) Logik der Forschung. Springer, Berlin.

Popper, K.R. (1963) Conjectures and Refutations. Routledge and Kegan Paul, U.K.

Popper, K.R. (1968) The Logic of Scientific Discovery. Hutchinson, U.K.

Popper, K.R. (1972) Objective Knowledge. Oxford University Press, U.K.

Popper, K.R. (1974) Replies to my critics. in The Philosophy of Karl Popper (ed. Schilpp, P.A.), Open Court.

Popper, K.R.(1983) Realism and the Aim of Science. Vol 1 of the Postscript. Hutchinson, U.K.

Porter, A. (1941?) The larval trematodes found in certain South African Mollusca with special reference to schistosomiasis (bilharziasis). Publication of the South African Institute of Medical Research No. XLII.

Price, E.W. (1931) Redescription on two species of trematode worms from the MacCallum collection, with a note on the family Pronocephalidae. Proc. U.S. Nat. Mus. 78: 1-10.

Putnam, H. (1962) What theories are not. in Logic, Methodology, and Philosophy of Science: Proceedings of the 1960 International Congress. Stanford University Press, California.

Rogers, D.J. and Appan, S.G. (1969) Taximetric methods for delimiting biological species. Taxon 18: 609-752.

Rohde, K. (1962) A new trematode *Diaschistorchis multitesticularis* sp. n. from a Malayan tortoise, Hieremys annandalei (Boulanger). J. Parasitol. 48: 296-297.

Rohlf, F.J., Colless, D.H. and Hart, G. (1983) Taxonomic congruence - a reanalysis. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Rosenberg, A (1985) The Structure of Biological Science. Cambridge University Press, U.K.

Ruiz, J.M. (1946) Pronocephalidae. Estudios das especies brasileiras e revisao da familia. Mem. Inst. Butantan 19: 249-372.

Russell, B. (1940) An inquiry into meaning and truth. Allen and Unwin, London.

Saxena, S.K. (1977) A collared monostome cercaria of the "ephemera" group of Sewell and its development in experimental definitive hosts. Excerpta Parasitol. en Mem. del Dr.Caballero y Caballero. Univ. Nat. Aut. Mexico.p.295-305.

Schuh, R.T. and Polhemus, J.T. (1980) Analysis of taxonomic congruence among morphological, ecological and biogeographic data sets for the Leptopodomorpha (Hemiptera). Syst. Zool. 29: 1-26.

Sharma, P.N. and Gupta, P.N. (1971) A new review of the genus *Neopronocephalus* Mehra, 1932 (Trematoda). Folia Parasitol. 18: 235-240.

Siddiqi, A.H. and Cable, R.M. (1960) Digenetic tematodes of marine fishes of Puerto Rico. N.Y. Acad. Sci. 17: 257-369.

Siddiqi, A.H. (1965) Two new trematodes from fresh water turtles in India. J. Helminth. 39: 277-280.

Simberloff, D. (1987) Calculating probabilities that cladograms match: a method of biogeographical inference. Syst. Zool. 36: 175-195.

Simpson, G.G. (1961) Principles of Animal Taxonomy. Columbia University New York.

Sinclair, N.R. (1972) *Parapronocephalum* Belopolskaia, 1952 (Trematoda): Notocotylid or Pronocephalid? A description of *Parapronocephalum reversum* sp. n. in shore birds. Proc. Helm. Soc. Wash. 39: 87-94.

Sneath, P.H.A and Sokal, R.R. (1973) Numerical Taxonomy. Freeman, San Francisco.

Sneath, P.H.A. (1983) Philosophy and method in biological classification. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Sokal, R.R. and Sneath, P.H.A. (1963) The principles of Numerical Taxonomy. Freeman, San Francisco.

Sokal, R.R. (1983) The phenetics-cladistics controversy: a personal view. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Sokal, R.R. (1983) Taxonomic congruence in the Caminalcules. in Numerical Taxonomy (ed. Felsenstein, J.). Springer-Verlag, Berlin.

Steele, E.J. (1980) Somatic Selection and Adaptive Evolution: on the inheritance of acquired characters. Williams and Wallace, London.

Stevens, P.F. (1980) Evolutionary polarity of character states. Ann. Rev. Ecol. Syst. 11: 333-358.

Strauch, J.G. (1984) Use of homoplastic characters in compatibility analysis. Syst. Zool. 33: 167-177.

Stuessy, T.F., and Crisci, J.V. (1984) Problems in the determination of evolutionary directionality of character-state change for phylogenetic reconstruction in Cladistics: perspectives on the reconstruction of evolutionary history (eds. Duncan, T and Stuessy, T.F.) . Columbia University Press, New York.

Suh, Y.Y., Soule, M. and Gorman, G.C. (1974) *Anolis* lizards of the Eastern Caribbean: a case study in evolution I. Genetic relationships phylogeny, and colonization sequence of the *Roquet* group. Syst. Zool. 23: 387-399.

Sullivan, J.J. (1976) *Parapleurogonius brevicaecum* gen. et. sp. n. from a freshwater turtle in peninsular Malaysia (Trematoda: Pronocephalidae). South-East Asian J. Trop. Med. 7: 540-542.
Suppe, F. (1977) The Structure of Scientific Theories. University of Illinois Press, Urbana.

Swofford, D.L. and Berlocher, S.H. (1987) Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36: 293-325.

Swofford, D.L. (1985) PAUP: Phylogenetic Analysis Using Parsimony Ver. 2.4.0 Manual. Illinois Natural History Survey, Illinois.

Threlfall, W. (1979) Three species of Digenea from the Atlantic leatherback turtle (*Dermatochelys coriacea*). Can. J. Zool. 57:1825-1829

Thapar, G.S. (1968) Studies on the life histories fo trematode parasites. I. A new monostome cercaria, *Cercaria Neopronocephalus indicus* (Ephemera group) from the snail *Melanoides tuberculatus* from Lucknow and its probable identity to *Neopronocephalus triangularis*. Ind. J. Helminthol. 20:125-131.

Vigueras, I.P. (1955) Contribucion al conocimiento de la fauna helminthologica cubana. Mem. Soc. Cub. Hist. Nat. 22: 21-71.

Watrous, L.E. and Wheeler, Q.D. (1981) The out-group comparison method of character analysis. Syst. Zool. 30: 1-11.

Whittaker, R. H. (1969) New concepts of kingdoms of organisms. Science 163:150-160.

Wiley, E.O. (1975) Karl R. Popper, systematics and classification: a reply to Walter Bock and other evolutionary taxonomists. Syst. Zool. 24: 233-243.

Wiley, E.O. (1981) Phylogenetics: the theory and practice of phylogenetic systematics. Wiley and Sons, New York.

Yamaguti, S. (1958) Systema Helminthum. Vol I. The digenetic trematodes of vertebrates. Interscience, New York.

Yamaguti, S. (1972) Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Co., Japan.

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